Original Article

In silico Design, and *In vitro* Expression of a Fusion Protein Encoding *Brucella abortus* L7/L12 and SOmp2b Antigens

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Abstract

Background: L7/L12 is a protective antigen conserved in main *Brucella* pathogens and is considered as potential vaccine candidate. Outer membrane protein 2b is an immunogen conserved in all *Brucella* pathogens. **Materials and Methods:** The purpose of the current study was to *in silico* design a L7/L12-SOmp2b fusion protein and *in vitro* production of the chimera. Two possible fusion forms, L7/L12-SOmp2b and SOmp2b-L7/L12, were subjected to *in silico* modeling and analysis. Cloning and expression of the fusion protein has been done in the pET28a vector and *Escherichia coli* Bl21 (DE3), respectively. **Results:** Analysis and validation of the fusion proteins three-dimensional models showed that both models are in the range of native proteins. However, L7/L12-SOmp2b structure was more valid than the SOmp2b-L7/L12 model and subjected to *in vitro* production. The major histocompatibility complex II (MHC-II) epitope mapping using Immune Epitope DataBase indicated that the model contained good MHC-II binders. The L7/L12-Omp2b coding sequence was cloned in pET28a vector. The fusion was successfully expressed in *E. coli* BL21 by induction with isopropyl-β-d-thiogalactopyranoside. The rL7/L12-SOmp2b was purified with Ni-NTA column. The yield of the purified rL7/L12-SOmp2b was estimated by Bradford method to be 240 μg/ml of the culture. Western blot analysis revealed a specific reactivity with purified rL7/L12-SOmp2b produced in *E. coli* cells and showed the expression in the prokaryotic system. **Conclusions:** Our data indicates that L7/L12-SOmp2b fusion protein has a potential to induce both B- and T-cell-mediated immune responses and it can be evaluated as a new subunit vaccine candidate against brucellosis.

Keywords: *Brucella, cloning, fusion protein, in silico design, L7/L12, outer membrane protein 2b*

Introduction

Brucellosis is the most common bacterial zoonotic disease infecting approximately half a million people annually around the world.[1,2] Abortion caused by the brucellosis imposes significant economic losses in livestock and the disease is known as a potentially life-threatening multi-system disease (undulant fever, arthritis, endocarditis and meningitis) in human.[3,4] The causative agent of Brucellosis, genus of *Brucella*, consists of more than ten species among them *Brucella abortus, Brucella melitensis* and *Brucella suis* cause most of the animal and human diseases.[1,2] *B. melitensis* is the cause of the most severe form of the disease in humans, following by *B. suis, B. abortus* and *Brucella canis* causing milder diseases. Immunization against *Brucella* infections in animals is usually performed by administration of the live attenuated smooth *Brucella* strains: *B. melitensis* strain Rev. 1, *B. abortus* strain S19, and *B. abortus* strain RB51.[5] However, these vaccines have major disadvantages of being pathogenic for humans, being resistant to streptomycin and interfering with the lipopolysaccaride based serological tests.[5,6] Although commercial vaccines are available for animal brucellosis, there is no effective and safe vaccine against human *Brucella* infections. Recently, with the purpose of developing effective human brucellosis vaccines, extensive studies have been performed to understand the mechanisms of protective immunity against *Brucella*.[7,8] Bioinformatics analyses showed that *Brucella* lacks classical bacterial virulence related sequences and genes, such as exotoxins, capsule, flagella, fimbriae, plasmids, lysogenic phage, antigenic variation, cytolysins, or pathogenic islands; making characterization of pathogenic mechanisms in this organism highly challenging.[4,9] Immunoproteomics considerably facilitated the identification

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of many potential immunogenic proteins in *Brucella*. However, only a few antigens have demonstrated important protective efficacy, *in vivo*.[10] Cell mediated mechanisms, particularly, Th1 immune responses characterized by the production of interferon-γ (IFN-γ) are crucial for the clearance of *Brucella* infection.[11] Studies on experimental and human brucellosis indicated that IFN-γ is the principal cytokine active against *Brucella* infection resulting from *Brucella* recognition by host immune receptors such as toll-like receptors which leads to interleukin-12 (IL-12) production.[2,4] A critical role of IFN-γ in the resistance to *Brucella* infection was demonstrated in mice model by *in vivo* antibody neutralization experiments and also by IFN-γ knock out mouse study.[12,13] However, the study done by Vitry *et al*. showed that IFN-γ-producing CD8+ T-cells or B-cell-mediated humoral immunity plays only a modest role in the clearance of bacteria during primary infection.[13] CD8+ cytotoxic T-lymphocytes are critical in killing *Brucella*-infected target cells.[12] Th1 cell-oriented responses against *Brucella* infection are best stimulated by live vaccines or protective proteins in presence of adjuvants favor cell-mediated immune mechanisms.[14,15,16]

The 13.5 kDa L7/L12 ribosomal protein of *B. abortus* which also exists in *B. melitensis* and other *Brucella* species has been identified as an immunodominant and protective antigen. The recombinant L7/L12 protein and DNA vaccine encoding L7/L12 gene can elicit strong cell-mediated immunity (Th1 responses) and promote protection against *Brucella* infection in BALB/c mice.[7,17-19] Moreover, it has been identified that the L7/L12 from *B. melitensis* and *B. abortus* is a major component in the antigenicity of Brucellin INRA (brucellergen) for delayed-type hypersensitivity in *Brucella*-sensitized guinea pigs.[17,20]

Brucella outer membrane protein 2b (Omp2b) is a 36 kDa porin existed in common *Brucella* pathogens and bioinformatics studies shows that it could be a priority antigen for designing a new subunit vaccine.[8,10] We have previously cloned and expressed the SOmp2b (a form lacking the signal peptide) protein and also the truncated form (aa163-aa362), successfully.[21,22] Our *in silico* analysis of Om2b protein indicated that the antigen is potential to induce both B- and T-cell mediated immune responses and it can be evaluated as a new subunit vaccine candidate against brucellosis. Moreover, further *in vivo* evaluation of the immunogenicity and protective efficacy of the SOmp2b and its truncated form (TOmp2b) done by us showed that SOmp2b and TOmp2b are potential vaccine candidates against *B. melitensis* and *B. abortus* infections. Three vaccine strategies based on the SOmp2b and TOmp2b antigens have been able to induce both homoral and Th1 immune responses and confer protection against *Brucella* infection in mice model.[22,23]

With regard to the fact that *B. melitensis* and *B. abortus* are the main cause of human brucellosis, design of a new vaccine candidate inducing protection against both pathogens would be ideal. Additionally, it has been revealed that using multivalent vaccine system increases the levels of both the protection and immune responses against *Brucella* infection.[15,20,24,25] In the present study, we aimed to design and express a fusion construct from SOmp2b and L7/L12 antigens. Further evaluation of the cross-protective efficacy of the fusion against *B. melitensis* and *B. abortus* is an ongoing project in our lab.

Materials and Methods

Strains, plasmids and media

The heat inactivated *B. abortus* strain 544 was purchased from the microbial collection of the Pasteur Institute of Iran (Karaj, Iran). The prokaryotic expression vector pET28a (Novagene, USA) and *Escherichia coli*, BL21 (DE3) (Stratagene, USA) were used for rL7/L12-SOmp2b protein production. Bacterial strains were cultured in LB broth or agar (Merck, Germany) containing 50 μg/ml of kanamycin, at 37°C. Unless otherwise mentioned in the text, all reagents, kits and enzymes used for amplification and cloning parts were from Themofisher, USA.

In silico design and modeling of the fusion

In silico design, modeling and structure validation of SOmp2b was performed as described previously.[21] Then L7/L12-SOmp2b and SOmp2b-L7/L12 proteins three-dimensional (3D) modeling was performed using Threading ASSEmbly Refinement (I-TASSER) server.[26]

Validation of the fusion three-dimensional model

Validation and analysis of the models were carried out using Protein Structure analysis (ProSa) server,[27] Swiss PDB Viewer version 4 software (Swiss Institute of Bioinformatics, Structural Bioinformatics Group, Biozentrum, Basel, Swiss) and Ramachandran Plot Analysis (RAMPAGE) server.[28] I-TASSER output provides the C-score (a confidence score for estimating the quality of predicted models by I-TASSER), TM score (a metric for measuring the global fold similarity of between the query and template protein) and RMSD (a metric for measuring the similarity in 3D structures of the query and template protein by root-mean-square deviation of atomic positions of the Cα atomic coordinates after optimal rigid body superposition). Z-score and energy plots are calculated using by ProSa server. The Z-score indicates overall model quality in comparison to the Z-scores of all experimentally determined protein chains in current Protein Data Bank. The energy plot shows local model quality by plotting energies as a function of amino acid sequence position and is representative of the error parts. The Ramachanran plot provided by RAMPAGE server shows the number of residues in favored, allowed and outer regions.

L7/L12-SOmp2b protein structure analysis

Fusion ProSa including calculation of the molecular weight, theoretical isoelectric point (pI), extinction coefficient, total number of positive and negative residues, half-life, instability index, aliphatic index and grand average hydropathy (GRAVY) has been done using ProtParam server.[29] The surface accessibility, hydrophilicity and antigenicity of the L7/L12-SOmp2b protein were predicted using IEDB resources.

Secondary structure prediction

The secondary structure of the L7/L12-SOmp2b protein was predicted using GOR4.[30]

T-cell epitope prediction

The human major histocompatibility complex (MHC) molecules epitope mapping was performed for the selected fusion model using Immune Epitope DataBase (IEDB) in order to find fusion MHC-II binding capacity. In the IEDB output page, the predicted epitopes of the human MHC-II alleles (HLA-DR, HLA-DQ and HLA-DP) within the protein sequence are listed based on their binding affinity. The epitopes' affinities are presented as percentage (low percentage equals good binder).[31]

Prediction of antigenic B-cell epitopes

BCpred server was used for prediction of continuous B-cell epitopes (20 mers) of the L7/L12-SOmp2b.[32] Prediction of discontinuous B-cell epitopes from 3D protein structure was performed using ElliPro antibody epitope prediction tool.[33]

Prediction of allergenicity of the proteins

Prediction of allergenicity of the fusion protein has been done using AlgPred tool.[34]

DNA extraction and polymerase chain reaction amplification

B. abortus 544 chromosomal DNA was extracted using DNA extraction kit (Roche, Germany) according to the manufacturer recommendation. The coding sequences of SOmp2b (KP071938) and L7/L12 (AQIS01006.1) were separately amplified using overlap primers (TAG Copenhagen, Denmark) and finally fused by overlap polymerase chain reaction (PCR). Briefly, the coding sequence of L7/L12 (375bp) was amplified using primers 1 and 2 [Table 1]. The coding sequence of the SOmp2b (1023bp) was amplified with primers 3 and 4 [Table 1]. Finally the fusion construct (1395bp) was amplified by primers 1 and 4 [Table 1]. All PCRs were performed in 50 µl total volume containing 500 ng of template DNA, 1 µM of each primer, 200 µM dNTP-Mix, 1X *pfu* buffer containing MgSO4 and 1 unit of 2.5U/µl *pfu* DNA polymerase. The amplification conditions were: Hot start at 95°C for 3 min, followed by 30 cycles of denaturation step at 94°C for 45 s, annealing step at 55°C for 45 s and extension step at 72°C for 1 min. The program followed by a final extension at 72°C for 7 min. The PCR products were analyzed by 1% agarose gel electrophoresis.

Cloning of L7/L12-SOmp2b in pET28a

The 1395bp PCR product of fusion was gel purified and then digested with Fast digest *Nhe I* and *Xho I* and then ligated to similarly digested ends pET28a using T4 DNA ligase at 4°C over night. Competent BL21 (DE3) strains of *E. coli* were prepared by calcium chloride method and were used for transformation of pET28a-L7/L12-SOmp2b plasmid. The transformed *E. coli* BL21 (DE3) was cultured on LB agar containing 50 µg/ml of kanamycin and was selected by screening the colonies on the media containing antibiotic. Suspected colonies were further analyzed by PCR, restriction enzyme digestion and sequencing.

Expression and purification of recombinant L7/L12-TSOmp2b

Recombinant L7/L12-SOmp2b protein expression was induced with different concentration of isopropyl-β-d-thi ogalactopyranoside (IPTG) (0.1, 0.2 and 0.4) in a culture of bacteria with an OD 600 of 0.6. Induced bacteria were incubated for 4 h at 37°C and finally harvested by centrifugation at 4000 rpm, 10 min, 4°C and stored at −80°C. The expressed fusion protein was then purified with Ni-NTA column using denaturing method (Genescript, USA) according to manufacturer instructions. Dialyzing of the purified protein was done against 5M urea, 3M urea, 1M urea and phosphate buffered saline (pH 7.2) at 4°C, over night. Identity of the purified recombinant L7/L12-SOmp2b protein was confirmed by 15% SDS-PAGE and Western blotting as described previously.[35] The quantity of the fusion protein was done by Bradford assay. The purified recombinant protein was stored at −20°C for further *in vivo* analysis of its immunogenicity and protective efficacy.

Results

Three-dimensional structure modeling and validation of the fusion protein

The fusion coding sequence was designed from the SOmp2b and L7/L12 with regard to the published L7/L12 and SOmp2b gene sequences.[27,35] The tertiary structure of fusion proteins was predicted using I-TASSER server [Figure 1a]. Validation of the 3D models was done using related bioinformatics tools. The results are summarized in the Table 2. The Z-score for both models showed that they were in the range of native proteins of similar size. The energy plot of residues indicated that C-terminal of the L7/L12-SOmp2b and N-terminal of the SOmp2b-L7/L12 were positive regarding the presence of Omp2b. However, the residue energies were more negative for L7/L12-SOmp2b model than those from SOmp2b-L7/L12 [Figure 1b]. With regard to the fusion protein structures analysis, the L7/L12-SOmp2b form was selected for further protein

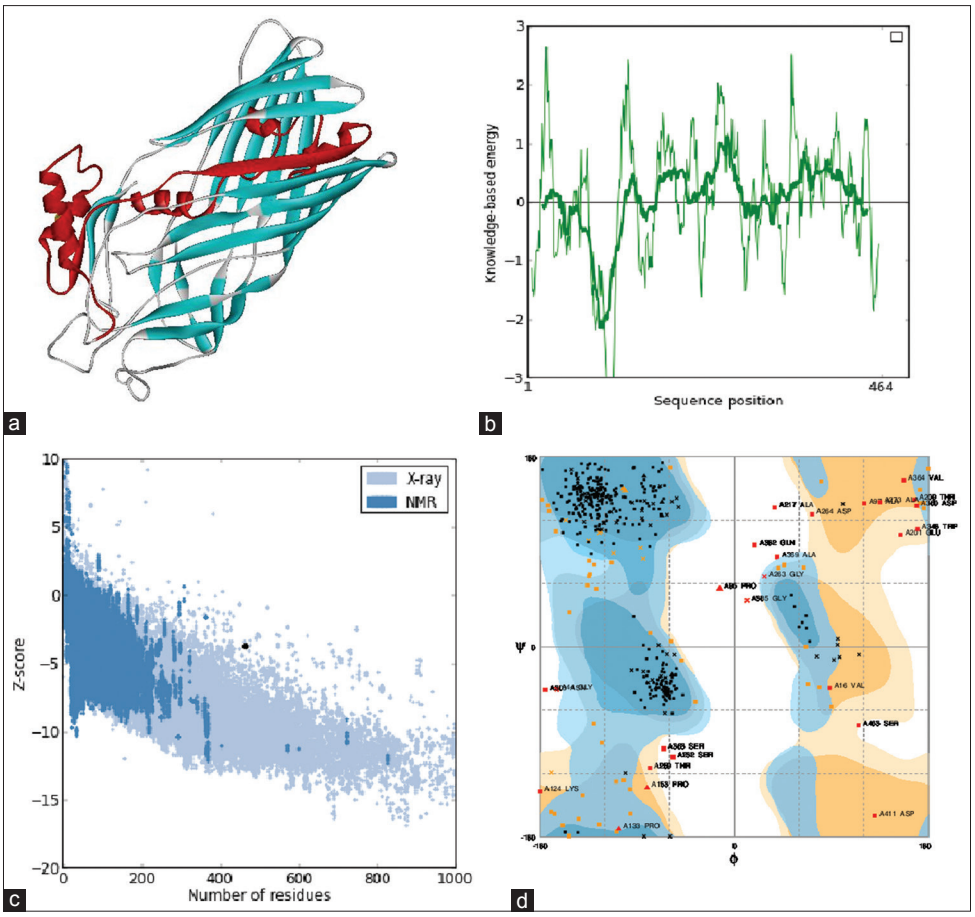

Figure 1: L7/L12-SOmp2b protein three-dimensional structure modeling and analyses results: (a) L7/L12-SOmp2b protein modeling. (b) The energy plot of protein residues. (c) The *Z*-score plot: The L7/L12-SOmp2bprotein structure is within the range of scores typically found for native proteins of similar size (the dot shows the location of the fusion protein). (d) Ranachandran plot

Table 1: Polymerase chain reaction primers for amplification of single and fusion coding sequences

Primer name	Primer sequence*	Primer features
Primer1	5'- CTAGCTAGCATGGCTGATCTCGCAAAGATC -3'	*Nhe I* restriction enzyme
Primer2	5'- ACGATTGCGTCGGCCTTGAGTTCAACCT -3'	
Primer3	5'- AGGTTGAACTCAAGGCCGACGCAATCGT -3'	*Xho I* restriction enzyme
Primer4	5'- CCGCTCGAGTTAGAACGAACGCTGGAAG -3'	

*The sites for restriction enzymes are underlined

Table 2: Three-dimensional models validation results

Fusion model	*C*-score	TM score	RMSD	*Z*-score
SOmp2b-L7/L12	−1.04	0.58	9.5	−2.42
L7/L12-SOmp2b	−1.38	0.54	10.4	−3.73

RMSD: Root-mean-square deviation, TM: Template modeling score

production [Figure 1]. Stability of the L7/L12-SOmp2b model was evaluated by the Ranachandran plot. Number of residues in favored, allowed and outer region was 82.7%, 11.9% and 5.4%, respectively.

Fusion protein structure analysis

Prediction of surface accessibility, hydrophilicity and antigenicity of the L7/L12-SOmp2b protein was performed using IEDB resource. The best scored peptides are summarized in Table 3. The molecular weight and the theoretical pI of the SOmp2b were 49.21 kD and 4.62, respectively. Total numbers of negatively and positively charged residues were 64 and 40, respectively. The half-life of the fusion protein was >10 h and >20 h in *E. coli* and *in vivo,* respectively. The instability index, Aliphatic index, and GRAVY were 22.65 (stable), 78.28, and −0.183, respectively.

Secondary structure analysis of L7/L12-SOmp2b

Fusion protein secondary structure prediction indicated that the protein consists of 33.62% (156 residues) alpha helix, 23.72% (198 residues) extended strand and 42.67% (156 residues) random coil [Figure 2].

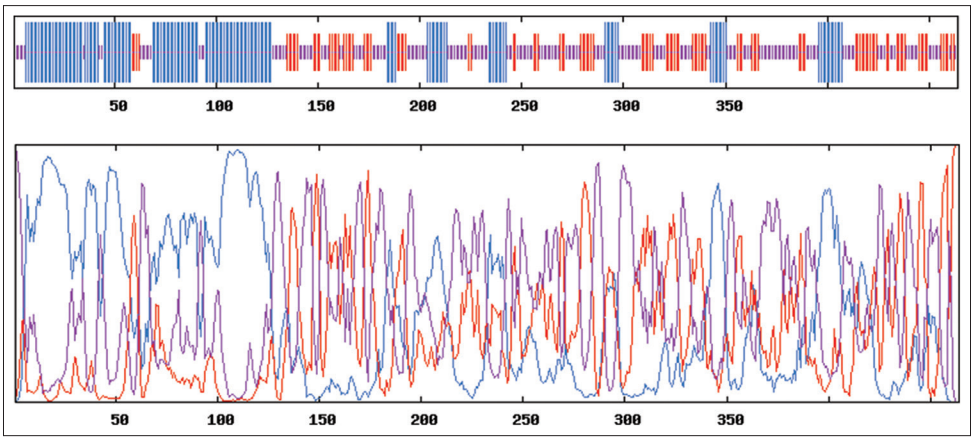

Figure 2: Secondary structure prediction of L7/L12-SOmp2b

Table 3: Protein structure analysis of L7/L12-SOmp2b using Immune Epitope DataBase resource

Parameter	Start-end	Peptide	Score
Surface accessibility	104-109	KDEAEK	5.489
	52-57	TPDQNY	4.411
	375-380	AEEKTE	4.1
Hydrophilicity	227-233	EQGGDND	7.457
	298-304	GNETSSG	6.343
	302-308	DNDGGYT	5.957
Antigenicity	136-142	VEYVRVC	1.207
	338-344	VVAYDSV	1.179
	421-427	VAYELVP	1.165

T-cell epitope prediction

The location of T-cell epitopes with strong affinity for human MHC-II alleles was predicted using IEDB resource. Top good binders for three HLA-II alleles are summarized in Table 4.

Antigenic B-cell epitope prediction

Identification of 20mer Linear B-cell epitopes was performed using BCpred server [Table 5]. Discontinuous (conformational) B-cell epitopes were predicted using Elipro tool [Table 6]. The best B-cell epitopes were selected according to the criteria based on cut-off values for BCpred and Elipro which were >0.8 and >0.5, respectively.

L7/L12-SOmp2b amplification and cloning

Gel electrophoresis of the PCR amplified L7/L12, SOmp2b [Figure 3a] and L7/L12-SOmp2b [Figure 3b] sequences showed main fragments of 375bp, 1023bp and 1395bp, respectively. The integrity of the constructed vector pET28-L7/L12-SOmp2b was confirmed by PCR, restriction digestion analysis and sequencing. Figure 3c shows the digestion results for positive clones. Recombinant plasmids showed two bands; representing 5.3 kb pET28a plasmid and the 1395bp fusion construct.

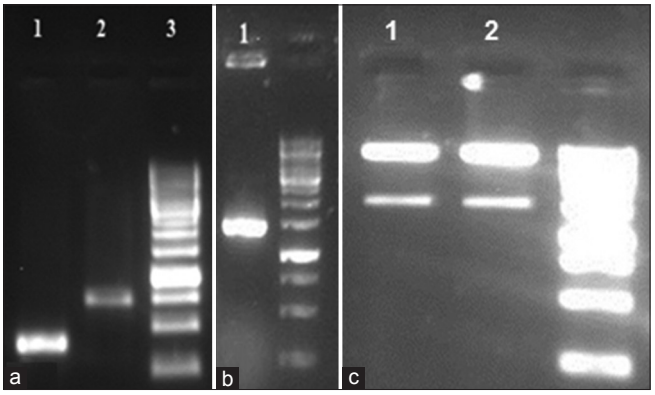

Figure 3: (a) L7/L12, SOmp2b amplification results. Lane 1: L7/L12 (375bp), lane 2: SOmp2b (1023bp), lane 3: 1 Kb DNA ladder (Fermentas). (b) L7/L12-SOmp2b amplification result: Lane 1: L7/L12-SOmp2b (1395bp), lane 2: 1 Kb DNA ladder. (c) Digestion results of recombinant plasmids. Lane 1 and 2: Recombinant plasmids; the digested pET28a plasmid (5300bp) and the fusion sequence (1395bp), lane 3: 1 Kb DNA ladder

Expression and purification of the rL7/L12-SOmp2b

The rL7/L12-SOmp2b protein was successfully expressed after induction with 0.1, 0.2 and 0.4 mM IPTG. The results of induction with different concentrations of IPTG were almost the same. Further, rL7/L12-SOmp2b protein was expressed with 0.2 mM IPTG, OD 600 = 0.6 for 4 h at 37°C. Expected rL7/L12-SOmp2b protein size of 49.21 kDa was detected after induction of the culture with IPTG. Most of it was found to be localized inside the inclusion bodies in the cells, after sonication. SDS-PAGE analysis of the induced and the purified rL7/L12-SOmp2b protein indicated the expected molecular mass of 49.21 kDa [Figure 4a]. The quantity of the purified fusion protein was estimated about 240 µg/ml of the culture. Western blotting with antibodies revealed the specific reactivity with purified rL7/L12-SOmp2b produced in *E. coli* cells [Figure 4b].

GenBank accession number

The coding sequence of L7/L12-SOmp2b was submitted in GenBank under accession number KX159298.

Table 4: Human leukocyte antigen Class II epitope prediction

HLA subtype	Start-end	Epitope	Score
HLADRB3*01:01	312-327	NYHIDGYMPDVVGGLK	0.01
HLADPA1*02:01/DPB1*05:01	203-217	GTLKTFTELRFNYAA	0.28
HLADQA1*05:01/DQB1*03:01	37-55	PVAVAAAGGAAPAAAEEK	0.01

*Low percentage=good binder. HLA: Human leukocyte antigen

Table 5: Prediction of B-cell linear epitopes By BCpred server

Position	Epitope	Score
298	EQGGDNDGGYTGTTNYHIDG	0.997
214	NYAANNSGVDGKYGNETSSG	0.996
375	TPDQNYGQWGGDWAVWGGLK	0.995
130	APEPEAVEYVRVCDAYGAGY	0.994
90	LVEGAPKAVKEGASKDEAEK	0.98
168	DVKGGDDVYSGTDRNGWDKS	0.977
273	AGSYRTGKISYTFTGGNGFS	0.965
426	VPGFTVTPEVSYTKFGGEWK	0.835
244	GGLRVGIDESEFHTFTGYLG	0.834

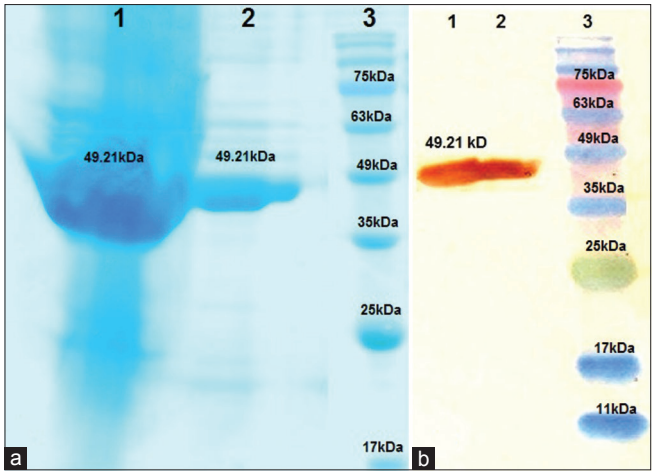

Figure 4: SDS-PAGE and Western blotting results of the purified fusion protein. (a) Lane 1: Induction result with 0.2 mM IPTG, lane 2: Purified fusion protein (49.21 kD), lane 3: Prestained protein ladder (Sinaclon Bioscience). (b) Lane 1: Purified Fusion protein (49.21 kD), lane 2: Prestained protein ladder (SinaClon Bioscience)

Discussion

Although many efforts have been performed to indentify new immunogens in *Brucella* proteome, not all of these new targets showed *in vivo* protective efficacy.[14,36,37] The *B. abortus* L7/L12 protein is considered as a potential target of subunit vaccines against brucellosis and some studies have concentrated on using this antigen as recombinant protein and DNA vaccine.[4,19,38,39] During the infection by *Brucella,* some of the bacterial structural components, such as the L7/L12 ribosomal protein, are involved in stimulating the immune response in the host. Recombinant L7/L12 protein could be detected as an antigen by sera from acute phase of brucellosis in infected human. Moreover, studies have shown that L7/L12 subunit vaccine can induce lymphocyte proliferation and elicit cellular immunity.[18,19,40,41] Absence of Omp2b from live attenuated *B. abortus* vaccine strain 19, studied by Bioinformatics analysis of the strain, indicates that Omp2b probably contributes to the attenuation of this mutant. Additionally, analysis of *Brucella* genome by Vaxign demonstrated that Omp2b is present in *B. abortus, B. melitensis*, *B. suis* and *B. canis* strains that are pathogenic to humans, but absent from *Brucella ovis* that is nonpathogenic to humans.[8] We have previously analyzed the immunogenicity and protective efficacy of *B. abortus* 544 Omp2b and L7/L12 proteins in BALB/c mice.[19,22,23] Our successful results encouraged us to design a chimera based on SOmp2b and L7/L12 immunogens.

In the current study, we aimed to design a fusion protein from L7/L12 and SOmp2b proteins using bioinformatics tools and express the fusion in the prokaryotic expression system. For this purpose, 3D modelling of two possible fusion forms is carried out using I-TASSER database generating the best 3D structure predictions among all automated servers.[26] The C-score and Z-score for both fusion models show that the proteins are with a correct topology and they are in the range of native protein conformations of the same size. The energy plot of the residues indicates that C-terminal of the L7/L12-SOmp2b and N-terminal of the SOmp2b-L7/L12 are positive due to the presence of Omp2b. This could be explained by the nature of the Omp2b as an exposed protein with high energy.[27] However, the residue energies are more negative for L7/L12-SOmp2b model than those from SOmp2b-L7/L12 indicating more stability of L7/L12-SOmp2b. Stability of the L7/L12-SOmp2b model was also evaluated by the Ranachandran plot indicating that 95% of the residues are located in the favored, allowed region.

Analyzing the physico-chemical parameters of the L7/L12-SOmp2b model protein using ProtParm tools demonstrates that the fusion is an acidic (pI: 4.62), stable (with instability index <40) and hydrophilic (negative hydrophathy values) protein. Regarding protein secondary structure prediction, L7/L12-SOmp2b protein structure consists of ten alpha-helixes that could play a major role in stability of the protein structure.

Regarding the World Health Organization recommendations, prediction of the potential allergenicity of therapeutic protein is realized as a significant parameter in vaccine development by.[34] Prediction of allergenicity of L7/L12-SOmp2b by AlgPred indicated that the construct is not an allergen.

Table 6: Identification of B-cell discontinuous epitopes using Elipro tool

Residues	Number of residues	Score
H256, T257, F258, T259, G260, Y261, L262, G263, D264, V265, I266, N267, D268, D269, V270, I271, S272, A273, G274, S275, Y276, R277, G279, D342, S343, V344, I345, E346, E347, W348, A349, Y395, Q396, A397, T398, Q399, K400, A401, A402, F403, V421, A422, Y423, E424, L425, V426, P427, G428, F429, T430, V431, T432, P433	53	0.806
V72, I73, K74, E75, R77, A78, L79, T80, G81, L82, G83, L84, K85, E86, A87, K88, D89, L90, V91, E92, G93, A94, P95, K96, A97, V98, K99, E100, G101, A102, S103, K104, D105, E106, A107, E108, K109, I110, K111, A112, Q113, L114, E115, A116, A117, G118, A119, K120, V121, E122, L123, K124, A125, D126, A127, I128, V129, A130, P131, E132, P133, E134, Y176, N219, S220, G221, V222, D223, G224, K225, Y226, G227, N228, E229, T230, S231, S232, G233, T234, V235, M236, E237, Q242, L243, G244, S292, A293, V294, A296, L297, E298, Q299, G300, G301, D302, N303, D304, G305, G306, Y307, T308, G309, T310, T311, N312, Y313, H314, I315, D316, G317, Y318, M319, P320, D321, V322, V323, G324, G325, L326, K327, G368, A369, Y370, S371, S372, A373, A374, T375, P376, D377, W412, G442, E443, E450, D451, N452, A453, W454, G455, G456, R459	141	0.699
V38, V40, A43, A47, A50, A51, A52, E53, E54, K55, T56, E57, F58, Y149, F150, Y151, I152, P153, G154, T155, E156, T157, C158, L159, V161, L192, V194, S195, T196, G197, S198, E199, T200, E201, L202, G203, T204, K206, F208, T209, G245, L246, R247, V248, G249, I250, E252, E254, F285, G288	50	0.636
Q361, F362, S363, V364	4	0.596

Since Th1 response mediated by MHC-II epitopes are indicated to be the significant key in defense against *Brucella* infection, the potential of the chimera to interact with the human HLA-II molecules is predicted using IEDB.[2,4,42,43] The location of the good binder peptides, presented by lower scores, is recognized in the fusion protein sequence. The prediction results indicate that the fusion is capable of inducing MHC-II molecules. Regarding [Table 2], top HLA-DR, HLA-DQ and HLA-DP binders are ^{312}NYHIDGYMPDVVGGLK^{327}, ^{203}GTLKTFTELRFNYAA^{217} and ^{37}PVAVAAA GGAAPAAAEEK^{55}, respectively. According to the prediction results, epitope ^{312}NYHIDGYMPDVVGGLK^{327} (score: 0.01) is the best T-cell epitope with the highest binding affinity for HLADRB3*01:01. Prediction of the protective continuous B-cell epitopes using BCpred tool resulted in identification of nine epitopes in which ^{298}EQGGDNDGGYTGTTNYHIDG ^{318}(score: 0.997) was predicted as the best linear B-cell epitope. These epitopes were previously predicted as the best T- and B-cell epitopes identified within the Omp2b protein sequence.[21] Besides, the location of the conformational B-cell epitopes is predicted using Ellipro tools. The results show that the best discontinuous B-cell epitope (score: 0.806) is located in the most hydrophilic region within the fusion protein structure. This strong epitope could be used in developing new *Brucella* immunodiagnostic tests.

Due to the significance of the high level production of recombinant protein in immunological studies, the fusion was cloned in pET28a expression vector. The pET system is the most powerful system yet developed for the cloning and expression of recombinant proteins in *E. coli*.[43] The plasmid construct pET28a-L7/L12-SOmp2b is transferred into *E. coli* BL21 (DE3) that contains T7 RNA polymerase and the expression is induced by the addition of IPTG. Successful induction of rL7/L12-SOmp2b expression by different concentrations of IPTG and high level production of the fusion protein in the prokaryotic system demonstrated the high efficiency of our fusion construct.

Conclusions

In the current study, a new vaccine target against *Brucell*a infection was designed with the help of bioinformatics tools. Our data indicates that L7/L12-Omp2b fusion protein has a potential to induce both B- and T-cell-mediated immune responses and it could be evaluated as a new subunit vaccine candidate against brucellosis. Further studies evaluating the immunogenicity and protection conferred by the rL7/L12-SOmp2b against *Brucella* challenge in mice model are underway in our lab.

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Conflicts of interest

There are no conflicts of interest.

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