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Research Paper

Design and In Silico Evaluation of BLS Scaffold-based Subunit Vaccine Candidates Incorporating L7/L12-Derived Epitopes Against Brucellosis

Narges Sahranavard[1*], Esmaeil Babaei[2]

1. Department of Biology, School of Natural Sciences, University of Tabriz, Tabriz, Iran.

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ABSTRACT

Introduction: *Brucella*, a facultative intracellular bacterium, is the causative agent of brucellosis in humans and livestock. Although traditional vaccine approaches against *Brucella* have been somewhat effective, they often come with unwanted side effects, since vaccines may also contain other bacterial components that are potentially pathogenic or may trigger adverse inflammatory responses. To overcome these limitations, recent research has increasingly focused on developing subunit vaccines. This strategy aims to identify and use *Brucella*-specific immunogenic components that can induce a strong and protective immune response while eliminating the pathogenic components of the bacterium.

Materials & Methods: The protein sequences of BLS and L7/L12 were obtained from NCBI, and the predicted epitopes of L7/L12 were retrieved from the IEDB server. The most suitable L7/L12 epitopes, including predicted B-cell and T-cell stimulatory epitopes that overlapped in some regions, were selected and inserted into non-conserved regions of the BLS protein. The VaxiJen server was used to assess the antigenicity of the sequence, and SWISS-MODEL was used to model the 3D structure of the recombinant protein. The Z-score index from ProSA-web was used to evaluate the quality of the generated model, and the Ramachandran plot was generated using the Ramplot server. Other indicators, including the non-allergenicity and non-toxicity of the recombinant protein as well as physicochemical properties such as stability and solubility, were also evaluated.

Results: The antigenicity of the BLS sequence increased after introducing the proposed epitopes, rising from 0.52 to 0.60 and 0.55 in models 1 and 2, respectively. The Z-scores of the proposed structures 1 and 2 in ProSA-web were −6.6 and −5.57, respectively. According to the Ramachandran plot, no amino acids were observed in the disallowed region in either

*** Corresponding Author:**
Narges Sahranavard
Address: *Department of Biology, School of Natural Sciences, University of Tabriz, Tabriz, Iran.*
Tel: *+98 (993) 4003453*
E-mail: *sahranavardn@tabrizu.ac.ir*

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models, or any occurrences could be ignored. Analysis of the recombinant sequence in the IEDB server revealed L7/L12 epitopes among the proposed epitopes. Other results confirmed the stability, suitable solubility, and non-allergenicity of the recombinant protein.

Conclusion: Based on these results, recombinant constructs based on the BLS protein containing L7/L12 epitopes are proposed as potential candidates for further development of brucellosis subunit vaccines.

1. Introduction

Brucellosis causes significant economic losses =in domestic animals due to abortion and infertility. In humans, the disease is associated with persistent infection and chronic inflammatory responses [1]. The primary causative agent of brucellosis in humans and livestock is the bacterium *Brucella*, a facultative intracellular pathogen. Transmission commonly occurs through ingestion or inhalation of *Brucella* organisms via the oral and nasal routes [2]. T- cell-mediated immune responses play a crucial role in controlling and eliminating *Brucella* infections [1]. The clinical significance of *Brucella* stems from its ability to persist and multiply within host cells, along with its efficient transmission between animals and humans [2, 3].

Conventional approaches to developing *Brucella* vaccines have primarily relied on whole bacterial strains, either inactivated or attenuated. Although effective, these vaccines have several limitations, including the potential to cause disease in humans, induce abortion in pregnant animals, and interfere with accurate diagnosis of infection in vaccinated animals [4, 5]. Because of these challenges associated with first-generation vaccines, developing safe and effective subunit vaccines is a desirable strategy for controlling brucellosis in humans and animals [5, 6].

Several immunogenic *Brucella* antigens have been evaluated as potential vaccine candidates, including lumazine synthase (BLS), ribosomal protein L7/L12, outer membrane proteins (Omp16, Omp19, Omp25, and Omp31), DnaK heat shock protein, periplasmic immunogenic protein Bp26, 39-kDa sugar-binding protein (p39), trigger factor (Tig), SurA peptidyl-prolyl cis/trans isomerase, and Cu/Zn superoxide dismutase (SodC) [4, 7, 8]. The incorporation of multiple protective antigens into a subunit vaccine formulation may enhance overall protective efficacy compared with the use of individual antigens alone [4, 6].

The *Brucella* ribosomal proteins L7 and L12 (L7/L12) are encoded by a single gene, with L7 generated from L12 through post-transcriptional modification at the N-terminal region [9]. Because of its strong immunogenic and protective properties, the L7/L12 protein has been identified as one of the major protective antigens of *Brucella* species. Previous studies have shown that incorporating the L7/L12 antigen into DNA vaccine platforms elicits robust cellular immune responses and provides protective immunity against *Brucella* infection in mice (Balb/c) [10, 11]. Another promising *Brucella* antigen is BLS, a bacterial enzyme encoded by a 477-bp gene that produces a protein with a molecular weight of approximately 17 kDa. The highly immunogenic *Brucella* lumazine synthase has the properties of an effective adjuvant, making it a useful protein carrier for vaccines.

Furthermore, BLS can induce both humoral and cell-mediated immune responses, underscoring its potential as a valuable component of subunit vaccine formulations [12]. The BLS protein is a stable dimer composed of pentamers with 10 binding sites at the N-terminus. Structural studies have shown that peptides or some proteins can be fused to this protein without disrupting its structure. Additionally, studies have demonstrated that BLS can activate cellular responses by presenting CD8+ T cell epitopes through the MHC I pathway, inducing an antigen-specific cellular response with interferon-gamma (IFN-γ) production and a strong and prolonged humoral response in the absence of an adjuvant [13]. Linear epitopes are short, continuous stretches of amino acid residues within the primary sequence of an antigen that are exposed on the molecular surface and can be recognized by the immune system [14]. These epitopes can stimulate both B-cell and T-cell responses and may therefore exhibit high immunogenicity and induce robust antibody responses [15]. In a previous study, dominant T- and B-cell epitopes derived from the *Brucella* outer membrane proteins Omp22, Omp19, and Omp28 were identified using bioinformatics methods and used to design a safe and effective multi-epitope vaccine candidate. The selected epitopes from these three proteins were linked using HEYGAALEREAG and GGGS linkers. In addition, carrier sequences were attached to the N- and C-terminal

regions of the vaccine construct through EAAAK linkers to enhance the structural stability and immunogenicity of the designed construct [3].

In another study, a reverse vaccinology approach was applied using Omp10, Omp25, Omp31, and BtpB as target antigens. To maintain proper protein folding, CTL, HTL, and B-cell epitopes were linked using AAY, GPGPG, and KK linkers, respectively [16].

Although previous studies have successfully designed multi-epitope vaccine constructs against *Brucella*, most approaches have relied on directly assembling epitopes with linkers. The use of a stable, native *Brucella*-derived protein scaffold to present selected immunogenic epitopes remains less explored. Therefore, in this study, the BLS protein was selected as a structural scaffold, and the L7/L12 protein sequence was used as a source for identifying immunogenic epitopes for the design of a subunit vaccine candidate against *Brucella*. The aim of this study was to develop recombinant vaccine constructs by integrating highly immunogenic L7/L12-derived epitopes into a structurally stable BLS scaffold. The designed constructs were subsequently evaluated using a series of in silico approaches to assess their physicochemical properties, structural stability, antigenicity, and potential suitability as vaccine candidates for further experimental investigation against brucellosis.

2. Materials and Methods

2.1. Protein sequence assembly and signal peptide identification

In the present study, the BLS antigen was selected as the structural scaffold, and selected T-cell and B-cell epitopes derived from the L7/L12 antigen were incorporated into the vaccine construct. The amino acid sequences of BLS and L7/L12 were retrieved from the NCBI database using the accession numbers AHW58136.1 and AAA56790.1, respectively. The signal peptide regions of both antigens were predicted using the SignalP web server (version 4.1), and the predicted signal peptide sequences were subsequently removed. For this analysis, the protein sequences of the respective antigens in FASTA format were used.

2.2. Bioinformatic prediction of epitopes

The IEDB server was used to predict B-cell and T-cell epitopes for each antigen [17]. Predicted epitopes with favorable antigenic properties were selected for further analysis. The predicted epitopes of the L7/L12 antigen were retrieved from the IEDB web server for incorporation into the scaffold. Epitopes presented by MHC class I molecules are generally short peptides, typically comprising 8–11 amino acids, due to the limited size of their peptide-binding groove. In contrast, MHC class II molecules possess a larger binding groove, allowing them to accommodate and present longer peptides ranging from 13 to 17 amino acids [18]. Accordingly, the peptide length was set to 9 amino acids for MHC class I epitope prediction and 15 amino acids for MHC class II epitope prediction.

Finally, the most suitable predicted B-cell and T-cell epitopes of the L7/L12 antigen were selected, with preference given to epitopes that exhibited overlapping regions between B-cell and T-cell epitopes.

2.3. BLAST analysis and identification of turn and non-conserved regions

The native three-dimensional structure of the BLS protein (PDB ID: 1DI0) was downloaded in PDB format from the Protein Data Bank (PDB). Turn regions were identified using the DSSP server by submitting the PDB ID (1DI0) and obtaining the secondary structure assignment in DSSP format. Subsequently, BLASTP analysis was performed using the NCBI database to identify non-conserved regions of the BLS protein suitable for inserting the selected epitopes.

2.4. Insertion of epitopes into the appropriate scaffold regions

The 21-amino-acid sequence DLVEGAPKAVKEGASKDEAEK (Table 1, line 4) was selected as the first epitope, and the 15-amino-acid sequence KIVEDLSALTVLEAA (Table 2, line 2) was selected as the second epitope. The predicted epitope sequences were chosen to provide suitable antigenicity and maximize overlap between B-cell and T-cell epitopes. The first epitope overlapped with the sequence listed in Table 2 (line 1), and the second epitope overlapped with the sequence listed in Table 2 (line 2). The two selected epitopes were virtually inserted into both the predicted turn regions and all identified non-conserved regions of the BLS scaffold. Based on the structural evaluation criteria described below, two non-conserved regions (highlighted in blue and green in Figure 1) were identified as the most suitable insertion sites, whereas the turn regions and the remaining non-conserved regions were considered less favorable.

Table 1. Proposed linear epitopes of the B-cell stimulatory L7/L12 antigen

Line	Epitope Sequence	Length
1	31 GVSAAAP 37	7
2	39 AVAAAGGAAPAAAAEEKTE 57	19
3	65 GGAN 68	4
4	89 DLVEGAPKAVKEGASKDEAEK 109	21
5	115 EAAG 118	4

2.5. Evaluation of physicochemical properties, antigenicity, and allergenicity of the models

The key physicochemical properties of the designed recombinant constructs, including molecular weight, theoretical isoelectric point (pI), instability index, aliphatic index, and grand average of hydropathicity (GRAVY), were evaluated using the ProtParam online server. The allergenicity and antigenicity of the designed constructs were predicted using the AllerTOP and VaxiJen servers, respectively.

2.6. Prediction of three-dimensional structures and quality assessment of the models

The three-dimensional structures of the designed models were predicted using the SWISS-MODEL server. The quality of the predicted models was evaluated using the Z-score obtained from ProSA-web, and Ramachandran plots were generated using the Ramplot server to identify the most suitable model among the predicted structures. Finally, the predicted models were superimposed onto the native structure (saved in PDB format) using Swiss-PdbViewer, and the root mean square deviation (RMSD) values were calculated.

3. Results

3.1. Physicochemical properties, antigenicity, and allergenicity of the designed models

The structural and immunoinformatics analyses supported the suitability of the selected insertion sites for epitope incorporation. The antigenicity score of the BLS sequence increased from 0.52 before epitope insertion to 0.60 and 0.55 in models 1 and 2, respectively. Physicochemical analysis showed that models 1 and 2 had molecular weights of 19,509.28 and 21,063.09 Da, theoretical isoelectric points (pI) of 6.25 and 5.98, aliphatic indices of 91.06 and 96.60, and GRAVY values of −0.002 and 0.069, respectively. Based on their instability indices, both models were classified as stable proteins. In addition, both models were predicted to be non-allergenic.

3.2. Three-dimensional structure prediction and quality assessment of the models

The quality of the predicted models was evaluated using the Z-score obtained from the ProSA-web server. The Z-score values were −6.60 and −5.57 for recombinant models 1 and 2, respectively. The black dots representing the recombinant models were located within the dark blue region of the ProSA plot, indicating that the designed models were within the range of native proteins and exhibited acceptable structural quality (Figure 1).

The two predicted structures were considered the most suitable models based on Ramachandran plot analysis. In model 1, 152 amino acid residues (98%) were located in the favored region, 3 residues (2%) were located in the allowed region, and no residues were observed

Table 2. The most suitable proposed T-cell stimulating epitopes of the L7/L12 antigen

Line	MHC	Epitope Sequence	Length	Epitope Description
1	Class I	89 DLVEGAPKA 97	9	Overlapping with the epitope I (B cell stimulator)
	Class II	91 VEGAPKAVKEGASKD 105	15	
2	Class I	6 KIVEDLSAL 14	9	Epitope II (overlapping)
	Class II	6 KIVEDLSALTVLEAA 20	15	

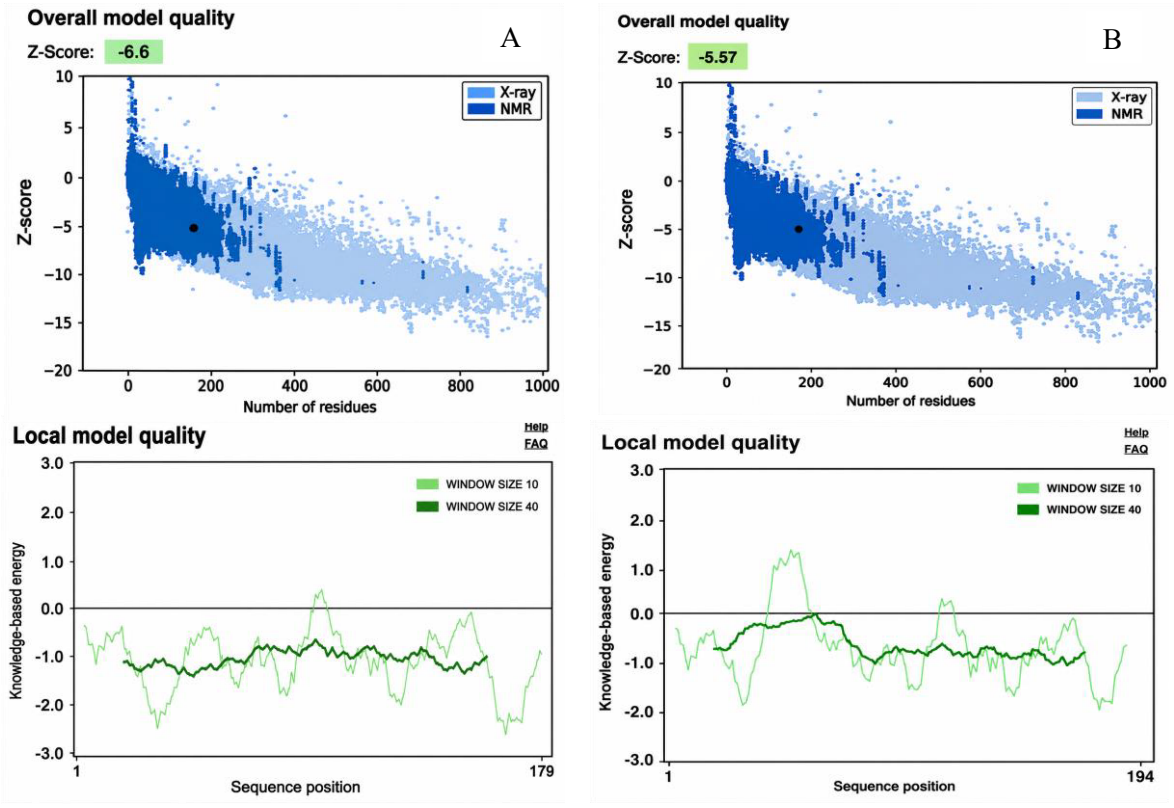

Figure 1. Z-Score index for models 1(A) and 2(B)

in the disallowed region. In model 2, only one residue (Asp65, 0.6%) was located in the disallowed region, which was considered negligible. In this model, 157 residues (95.7%) were located in the favored region, while 6 residues (3.7%) were located in the allowed region. Residues located in the favored and allowed regions exhibited suitable conformational properties and were consistent with those of native proteins based on their energy profiles and ψ/φ dihedral angles (Figure 2).

The RMSD values obtained by superimposing models 1 and 2 onto the native structure were 0.74 Å and 0.80 Å, respectively. These values fall within the acceptable range (0–2 Å), indicating a high degree of structural similarity between the predicted models and the native structure. Therefore, both models showed close struc-

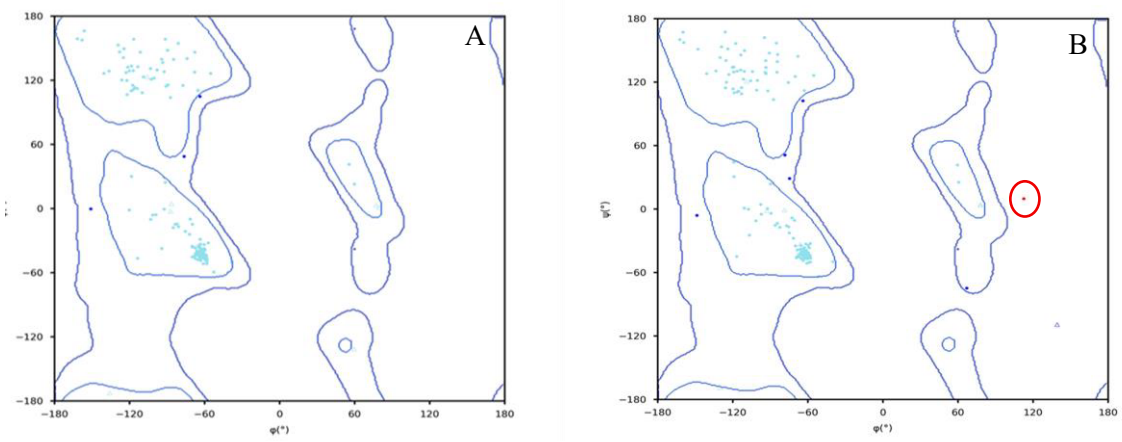

Figure 2. Ramachandran plot of models 1(A) and 2(B)

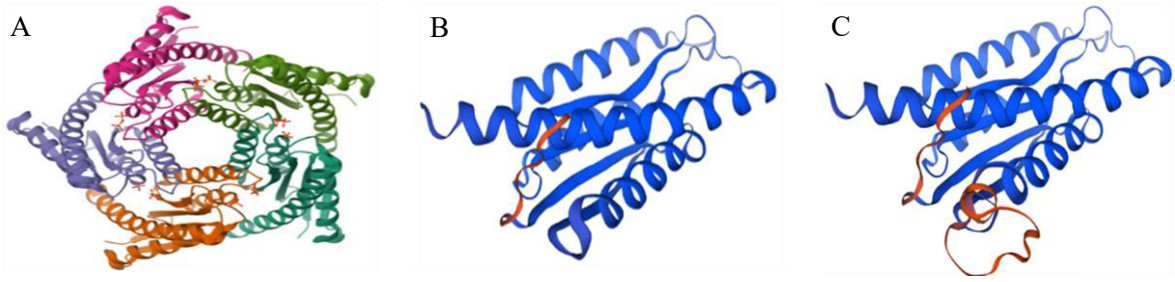

Figure 3. Spatial structure of native state (A), model 1(B) and 2(C)

tural conformity to the native state. Figures 3 and 4 show the individual structures and their superimpositions respectively.

3.3. Prediction of B-cell and T-cell stimulating epitopes in the designed models

To determine whether the inserted epitopes were retained as predicted B-cell and T-cell stimulating epitopes in the designed constructs, the sequences of models 1 and 2 were submitted to the IEDB server. The analysis showed that the selected B-cell and T-cell stimulatory epitopes derived from the L7/L12 protein were identified as predicted epitopes in both designed models (Tables 3 and 4).

Comparison of the predicted linear B-cell epitopes of the BLS antigen before and after epitope insertion revealed that, after incorporation of the first epitope into the BLS scaffold, the sequence DLVEGAPKAVKEGASKDEAEK corresponding to the first epitope, was identified within one of the predicted linear B-cell epitopes in both models. After insertion of the second epitope into the BLS scaffold, the sequence KIVEDLSALTVLEAA corresponding to the second epitope, was identified as one of the predicted linear B-cell epitopes in model 2 (Table 3). Additionally, a portion of the first epitope sequence was identified as a predicted T-cell stimulatory epitope in both models 1 and 2, whereas part of the second epitope sequence was identified as a predicted T-cell stimulatory epitope only in model 2 (Table 4).

Interestingly, although the second epitope sequence, KIVEDLSALTVLEAA, was not predicted as a linear B-cell stimulatory epitope in the original L7/L12 protein sequence, it was identified as a predicted linear B-cell stimulatory epitope after incorporation into the BLS scaffold in model 2 (Tables 2 and 3).

As shown in Table 3 and Figure 5, four B-cell stimulatory epitopes were predicted for the BLS antigen in the native sequence and the two designed models. In model 1, insertion of the first epitope (DLVEGAPKAVKEGASKDEAEK) increased the first (leftmost) peak of the antigenicity profile from 0.55 to 0.63. Incorporation of this epitope also slightly affected the second peak from the left, where the G epitope in Line 1 was changed to GGS in model 1 (Table 3). After insertion of the sec-

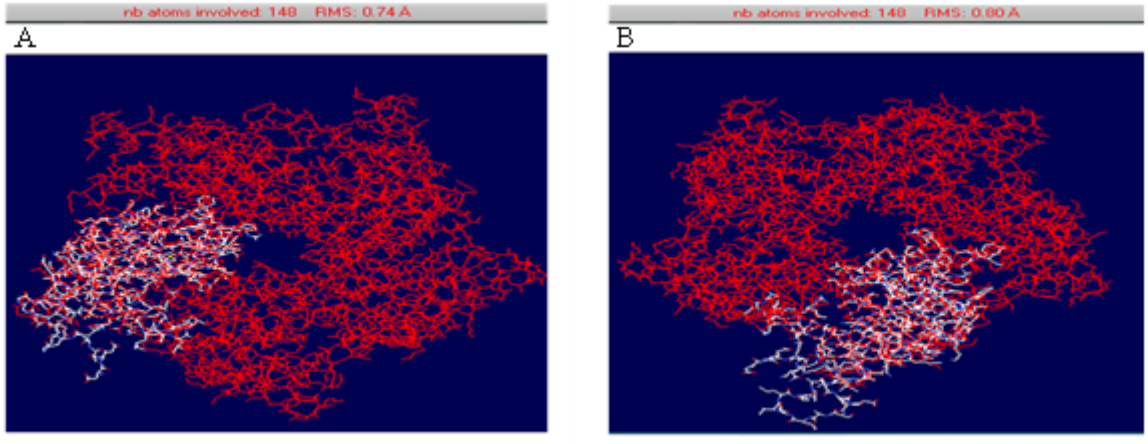

Figure 4. Superimpose models 1(A) and 2(B) on the native model

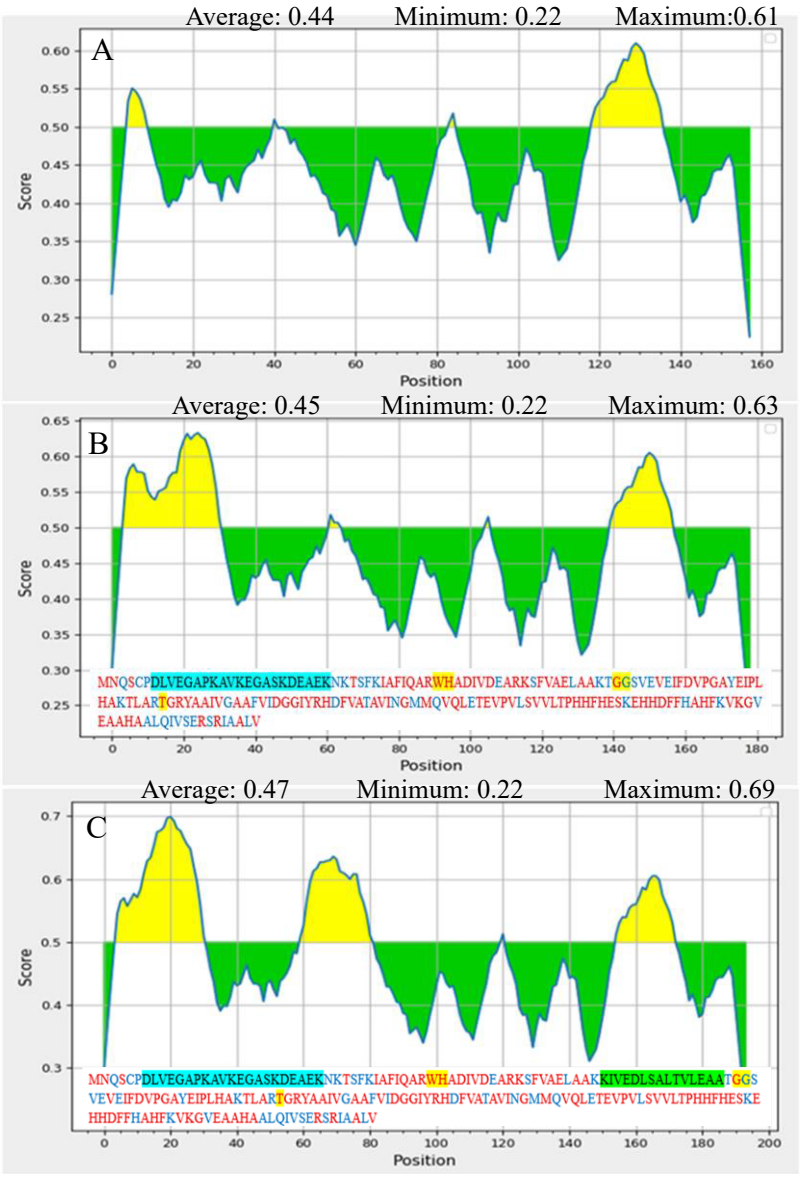

Figure 5. Predicted linear B-cell stimulating epitopes

A) BLS sequence (before epitope insertion), B) Model 1, C) Model 2

ond epitope (KIVEDLSALTVLEAA) and generation of model 2, the values of the first and second peaks from the left increased to 0.69 and 0.63, respectively.

4. Discussion

Accurate immunoinformatics approaches provide an effective strategy for the rational design of recombinant vaccine candidates by enabling the identification and incorporation of immunogenic regions of pathogens. Compared with conventional vaccine development approaches, these methods can reduce experimental costs and time by prioritizing promising vaccine candidates before laboratory evaluation [19, 20]. In the present study, immunoinformatics-based analyses were used to identify immunogenic epitopes derived from the L7/L12 antigen of *Brucella* and incorporate them into the BLS scaffold to design two recombinant vaccine models.

The physicochemical characteristics of the designed models suggested their potential stability and suitability as recombinant vaccine constructs. The theoretical isoelectric points of models 1 and 2 were 6.25 and 5.98, respectively, suggesting that both constructs are slightly acidic under neutral conditions. The aliphatic index is an important parameter associated with protein thermo

Table 3. Predicted linear B-cell stimulating epitopes of the BLS antigen

Line	Model / Without Epitope	Epitope Sequence	Length
1	BLS before epitope insertion	5 CPNKT 9	5
		41 G 41	1
		84 IY 85	2
		119 FHESKEHHDFFHAHFKVK 136	18
2	Model 1	4 SCPDLVEGAPKAVKEGASKDEAEKNKTS 31	28
		62 GGS 64	3
		105 IY 106	2
		140 FHESKEHHDFFHAHFKVK 157	18
3	Model 2	5 CPDLVEGAPKAVKEGASKDEAEKNKTS 31	27
		60 KKIVEDLSALTVLEAATGGSVE 81	22
		121 Y 121	1
		155 FHESKEHHDFFHAHFKVK 172	18

stability and reflects the relative volume occupied by aliphatic side chains, including alanine, valine, isoleucine, and leucine residues [21]. The elevated aliphatic index values observed for the designed models suggest that these proteins may maintain structural stability across a range of temperatures. Furthermore, the low GRAVY values obtained for both models indicate a higher tendency toward hydrophilic characteristics, which may contribute to improved solubility and proper folding of the recombinant proteins [21, 22]. Both designed models were predicted to be non-allergenic and showed increased antigenicity compared with the native BLS protein. Antigenicity is the ability of an antigen to induce an immune response, whereas allergenicity refers to the potential of a molecule to trigger undesirable immune reactions [23]. The increased antigenicity scores after epitope incorporation suggest that the inserted L7/L12-derived epitopes may enhance the immunogenic potential of the BLS scaffold. Moreover, the instability indices of both models were below 40, indicating that the designed constructs are theoretically stable proteins [24].

Structural evaluation further supported the quality of the designed models. Ramachandran plot analysis showed that most residues in both models were located within favored and allowed regions, indicating accept-

Table 4. Predicted T-cell stimulating epitopes of models 1 and 2

Line	Model (Structure)	MHC	Epitope Sequence	Length
1	1	Class I	7 DLVEGAPKA 15	9
			8 LVEGAPKAV 16	9
		Class II	6 PDLVEGAPKAVKEGA 20	15
2	2	Class I	7 DLVEGAPKA 15	9
			8 LVEGAPKAV 16	9
			61 KIVEDLSAL 69	9
		Class II	6 PDLVEGAPKAVKEGA 20	15
			61 KIVEDLSALTVLEAA 75	15

able stereochemical properties and structural reliability. Similar findings have been reported in previous studies in which designed multi-epitope vaccine constructs demonstrated satisfactory structural characteristics based on Ramachandran analysis and other structural evaluations [3, 16].

Analysis of the predicted B-cell and T-cell epitopes showed that the inserted L7/L12-derived sequences were retained as immunogenic regions within the designed constructs. Moreover, the increased height of the predicted antigenicity peaks after epitope insertion (Figure 1) suggests that incorporation of the selected epitopes enhanced the immunogenic profile of the BLS scaffold. Previous studies have also reported that BLS contains immunogenic regions, supporting its potential application as a vaccine scaffold [25].

In the present study, BLS was selected as the structural scaffold because of its stable decameric organization, intrinsic immunogenic properties, and capacity to accommodate foreign epitopes while maintaining structural integrity. The favorable physicochemical, structural, and immunoinformatics results obtained here support the suitability of BLS as a promising scaffold for developing recombinant vaccine candidates against *Brucella*. However, further experimental investigations are needed to validate the immunogenicity and protective efficacy of the designed constructs.

5. Conclusion

Based on the findings of this study, the chimeric constructs comprising the BLS scaffold and L7/L12-derived epitopes may represent promising candidates for the future development of subunit vaccines against brucellosis. The favorable physicochemical, structural, and immunoinformatics characteristics of these constructs support their potential as vaccine candidates. However, further in vitro and in vivo studies are required to validate their immunogenicity and protective efficacy.

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Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors' contributions

All authors contributed equally to the conception and design of the study, data collection and analysis, interception of the results and drafting of the manuscript. Each author approved the final version of the manuscript for submission.

Conflict of interest

The authors declared no conflict of interest.

Data availability

The authors confirm that the data supporting the findings of this study are available within the article and its references.

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