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Original Article

Bioinformatics analysis of T- and B-combined epitopes of OMP31 protein of *Brucella melitensis* in Xinjiang, China

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Abstract: Brucellosis is one of the major zoonoses in the word that widely inflicts health problems in animal and human. In China, brucellosis is gradually increasing in recent years, especially in the Xinjiang. Therefore, this study is to predict *Brucella* T-and B-combined epitopes of OMP31 protein by bioinformatics software. B-cell epitopes of OMP31 protein is predicted using DNAStar and IEDB software. The prediction of T-cell epitopes of OMP31 protein is performed by SYFPEITHI and ProPred MHC Class-II Binding Peptide Prediction Server. The results suggest that some position of OMP31 protein has several higher antigen indexes. The high-scoring of dominant B-cell epitopes were located at positions 25-31, 108-114, 201-207, 152-157, 166-172, 230-238, 123-127, 96-102 and 171-176. Five potential of dominant T-cell epitopes regions were revealed, located at positions 19-30, 92-106, 106-114, 137-179, 196-228. Finally, OMP31 protein has selected the three advantages of T- and B-combined epitopes, respectively are: 108-114, 152-157, 171-176. The bioinformatics research of OMP31 protein and T- and B-combined epitopes laid to the foundation of dominant epitopes vaccine.

Keywords: *Brucella*, bioinformatics, T-and B-combined epitopes, vaccine

Introduction

Brucellosis is a zoonotic chronic infectious disease caused by *Brucella*. *Brucella* is Gram-negative intracellular pathogenic bacteria that infect a range of different mammals including human [1-3]. *Brucella* infection in human can cause a broad range of clinical manifestations that most commonly result in sacroiliitis as well as spondylitis and peripheral arthritis [4, 5]. The incidence of brucellosis rose sharply in many countries. At present, approximately 500,000 new brucellosis cases are reported annually worldwide [6]. The infection is endemic in Mediterranean, the Middle East, central Asia, Africa and parts of Latin America [7]. In China, brucellosis has a wide distribution in many provinces especially in Xinjiang with higher prevalence of husbandry animals. Previous study revealed that brucellosis has significantly increased in Xinjiang [8]. Because it causes a wide variety of signs and symptoms, which is hard to diagnosis is immediately and patients may not receive the proper treatment in time. It is impossible to cure the patients when the acute brucellosis turns into a chronic phase [9]. Therefore, early preventive measures of *Brucella* infection are particularly important.

Vaccination has been suggested as the best strategy to prevent an infection from *Brucella* [10]. Hence, numerous *Brucella* vaccines have been developed and measured over the past several decades. At present, live attenuated vaccines are the most effective and are used worldwide for prevention from brucellosis. The research on *Brucella* antigen protein found that *Brucella* outer membrane protein is one of the main virulence factor and the most important antigen serology diagnosis [11, 12]. Tiwari et al [13] found that the OMP28 and OMP31 proteins of *Brucella* can help to diagnose brucello-

sis in clinical examination and serological diagnosis. Bowden et al [14] found that OMP31 has a good immunogenicity which can be used as a subunit vaccine candidate molecule for brucellosis. Wang et al [15] had predicted the *Brucella melitensis* BP26 and OMP31 proteins and founded the five T-cell antigen epitopes of BP-26 and five specific antigen epitopes of OMP31. Ghasemi et al [16] predicted the epitopes of BP26 in 93-111 and OMP31 in 48-74. The study revealed that the antigen epitopes of OMP31 as *Brucella* outer membrane proteins can be used for the development of *Brucella* subunit vaccine candidate molecules.

In summary, this study predicted and analyzed the T- and B-cells epitopes of OMP31 protein by bioinformatics software, especially its T- and B-combined epitopes, which may help develop a new vaccine to protect the host against *Brucella.*

Materials and methods

Reagents

OMP31 protein amino acid sequence: Released from GenBank in length of 240 bp of OMP31 protein of *B.melitensis*, the amino acid sequence is as the following:

MKSVILASIAAMFATSAMAADVVVSEPSAPTAAPVDTFSWTGGYIGINAGYAGGKFKHPFSSFDKEDNEQVSGSLDVTAGGFVGGVQAGYNWQLDNGVVLGAETDFQGSSVTGSISAGASGLEGKAETKVEWFGTVRARLGYTATERLMVYGTGGLAYGKVKSAFNLGDDASALHTWSDKTKAGWTLGAGAEYAINNNWTLKSEYLYTDLGKRNLVDVDNSFLESKVNFHTVRVGLNYKF.

Secondary structure prediction and analysis

The secondary structure of the OMP31 protein was predicted by the improved self-optimized prediction method (SOPMA) software (http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html). The inputs of the amino acid sequence of the OMP31 protein, and four conformational states, including helices, sheets, turns and coils, were analyzed.

Tertiary structure prediction and analysis

OMP31 protein tertiary structure prediction and analysis was conducted by online PHYRE2 Protein Fold Recognition Server and Swiss model (http://swissmodel.expasy.org/interactive). RasMol version was used to analyze different modes of the tertiary structure. The tertiary structure was displayed in the modes of Cartoon, Structure and Group.

T-and B-combined epitopes prediction and analysis

The software which predict B-cell epitopes are DNAStar (V5.0) (http://www.dnastar.com) and the online prediction software IEDB (http://tools.immuneepitope.org/bcell). The major histocompatibility complex class-II (MHC-II): human leukocyte antigen (HLA)-DRB1*0301-restricted T-cell epitopes were predicted using online prediction software SYFPEITHI (http://www.syfpeithi.de) and ProPred MHC Class-II Binding Peptide Prediction Server (http://www.imtech.res.in/raghava/propred/index.html).

Using Gamier-Robson [17] and Chou-Fasman [18] method of DNAstar software, separately predict Alpha helix and beta Angle of OMP31 protein; Using Emini method of DNAStar software [19], according to the specific composition of amino acid residues, predict the OMP31 protein molecular surface accessibility; Using Kyte-Doolittle [20] method of DNAStar software, according to the OMP31 amino acid molecules composition, predict its hydrophobic region and hydrophilic region; Using Karplus-Schulz [21] method of DNAStar software predict the OMP31 polypeptide skeleton flexibility; Using Jameson-Wolf [22] method of DNAStar software finish the prediction of antigenic determinant of OMP31 protein. IEDB [23] software is used for prediction and analysis of OMP31 protein, B-cell linear epitopes, beta corner, surface accessibility, flexibility of skeleton, antigen index, and hydrophobicity. SYFPEITHI [24] and ProPred MHC Class-II Binding Peptide Prediction Server [25] are used for comprehensive prediction and analysis of OMP31 protein and dominant T-cell epitopes.

Results

Prediction of the secondary structure of OMP31 protein

In order to assess the antigenic features of the OMP31 protein, we predicted its signal peptide using Signal-4.1 Server. S score is the signal peptide score, which suggesting the position of

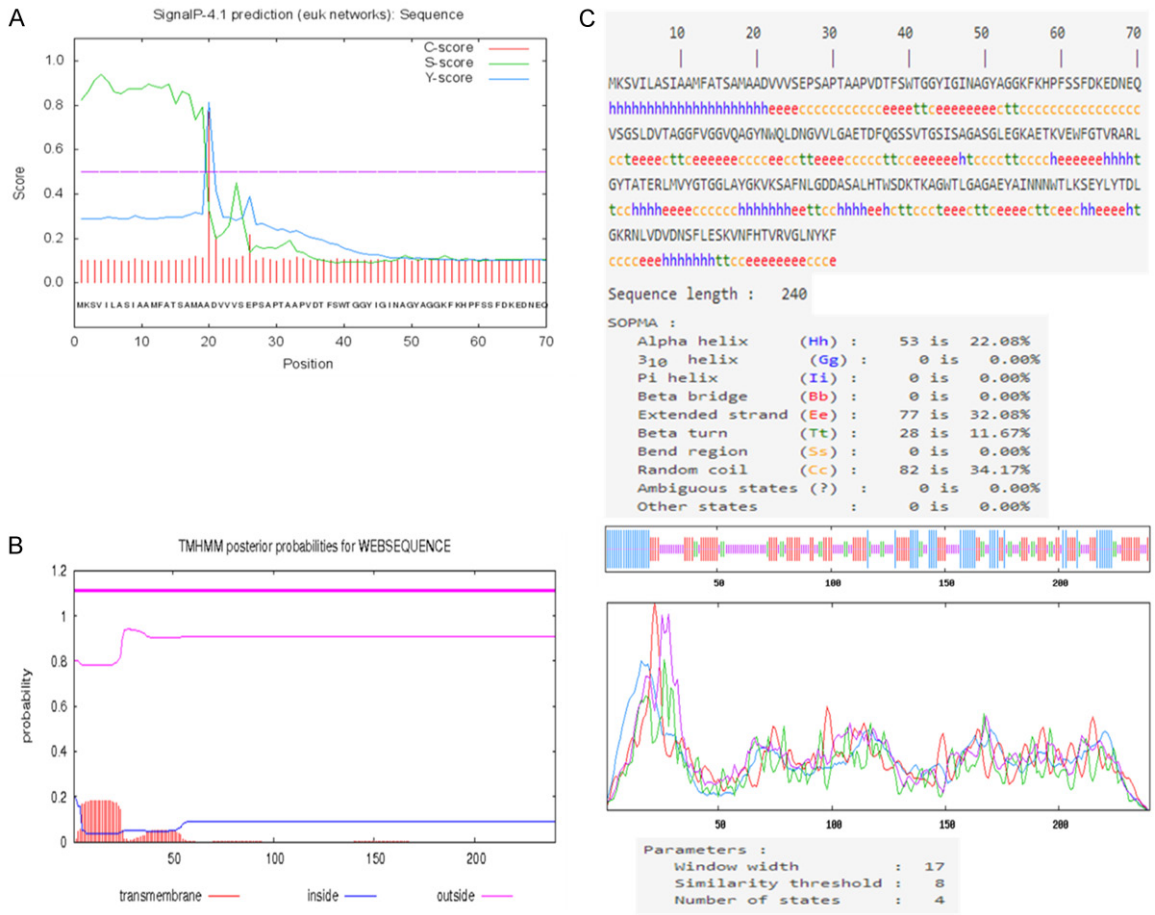

Figure 1. Secondary structure prediction results for the OMP31 protein. A. Signalp-4.1 Server software was used to analyze the signal peptide. B. The location of Amino acids was predicted by TMHMM software. C. The second structure was displayed by SOPMA.

signal peptide. Each amino acid has an S and C value, the signal peptide of is high, and the cleavage sites of C value are higher. The Y score distinguishes between C score peaks by choosing the one where the slope of the S score is steep. We speculated that there is a signal peptide in OMP31 protein, and signal peptide site is 1-19. When OMP31 protein matured, this signal peptide site will be removed. In order to improve the confidence level of the prediction results, we abandoned the signal peptide site (**Figure 1A**). Transmembrane and non-transmembrane regions of OMP31 protein were predicted by TMHMM server v 2.0. The region of amino acids that start from 19 to 240 was an extracellular domain (**Figure 1B**), its secondary structure was predicted by SOPMA Server software. The results revealed that the proportion of β turns, random coils, α helices and extended strands (β folds) accounted for 11.67%, 34.17%, 22.08% and 32.08% of the secondary structure, respectively (**Figure 1C**).

Results of tertiary structure prediction of OMP31 protein

To obtain a better understanding of its conformational structure, the tertiary structure of OMP31 protein was analyzed by Swiss-model. The tertiary structure was presented in the front and back view (**Figure 2A** and **2B**), the front and back view of Srick and group model (**Figure 2C** and **2D**), the front and back view of Structure and space-filling model (**Figure 2E** and **2F**), respectively. In the Structure mode, the amino acids in yellow area corresponded to the flexible regions (flexible areas are folded easily to form antigen epitopes). The front and back view of the tertiary structure showed that flexible regions in the laminated structure of the protein. The front and back view of Srick and group mode showed that magenta area corresponded to Alpha helix and pale blue is equivalent to beta Angle. Alpha helix and beta fold chemical bond is higher, is formed relative-

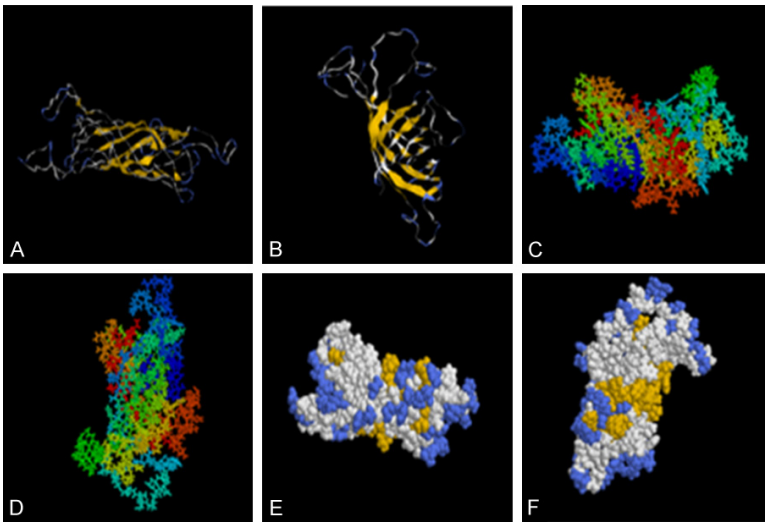

Figure 2. (A, B) Represents Tertiary structure prediction results of OMP31, the α helixes are shown by the curved lines and the β folds are shown by the laminated structures. The structure of the OMP31 modeled using Phyre, analyzed by Rasmol software in Srick and group (C) the front view (D) the back view. The structure of the OMP31 modeled using Phyre, analyzed by Rasmol software in Structure and spacefill (E) the front view (F) the back view. Magenta area corresponded to the α helixes, pale blue area corresponded to the turns. The laminated structures and the amino acids in the yellow area corresponded to the flexible regions of the protein.

ly fixed and often in the interior of the protein, is not easy to combine with antibodies and is not considered as an antigen epitope. While beta Angle and random curl at the surface of the protein, loose structure is easy express on the surface of the protein, is easy to combine with antibodies and more likely to become the antigen epitopes. The front and back view of the tertiary structure using space-filling model showed that the yellow region was gathered and distributed substantially at the surface of the structure. When an antibody binds an antigen, the antigen epitopes need to be fully exposed to facilitate better binding. Thus we speculated that the yellow and pale blue region in this model is the antigen epitopes that potentially binds to the antibody.

T-cell epitopes prediction of OMP31 protein

In order to improve the accuracy of prediction, we predicted the T-cell epitopes with SYFPEITHI and ProPred MHC Class-II Binding Peptide Prediction Server. HLA molecule selected type: HLA-DRB1*0301. We are using SYFPEITHI software to predict T-cell epitopes of OMP31 protein. The regions with high scores are 95-110, 212-227, 79-94, 83-98, 164-179, 2-17, 15-30, 147-162, 163-178, 203-218, 211-226, 213-228, 89-104, 61-76, 145-160, 158-173, 58-73, 137-152, 196-211 and 218-233 (**Figure 3A**). ProPred MHC Class-II Binding Peptide Prediction Server software is used to predict T-cell epitopes of OMP31 protein. The regions with high scores are 4-12, 19-26, 92-100, 106-114, 22-30, 98-106 (**Figure 3B**).

B-cell epitopes prediction of OMP31 protein

Using DNAstar software predicted B-cell epitopes: Using DNAstar software to predicted alpha helices of OMP31 protein, we found that alpha helices are located at positions 19-25, 66-71, 104-105, 121-135, 143-151, 167-178, 209-210 and 220-229. The beta corner are located at positions 20-26, 31-39, 44-50, 55-59, 73-94, 96-103, 110-117, 135-140, 146-166, 184-187, 192-195, 202-208, 212-219 and 226-240. In alpha helices and beta corner regions, the distribution of different seventeen corner regions and nine coil regions, corner regions are mainly distributed in: 26-29, 36-43, 48-55, 58-65, 67-74, 78-81, 89-92, 94-97, 106-109, 118-121, 152-155, 168-171, 176-180, 188-191, 196-199, 211-214, 218-221. coil regions are mainly distributed in: 28-30, 61-65, 109-111, 118-120, 140-142, 181-183, 188-190, 199-201, 236-237 (>1) (**Figure 4A**). Emini method of analysis showed that the surface probability plots were located at positions 62-69, 123-127 and 175-180 (**Figure 4A**). Kyte-Doolittle method of analysis showed that the hydrophilicity plot are located at positions 50-70, 85-95, 101-110, 118-131, 162-182, 190-217 and 218-228 (≥0.5) (**Figure 4A**). Karplus-Schulz method of analysis showed that the flexible regions are located at positions 25-31, 40-42, 53-57, 61-75, 94-96, 102-115, 119-128, 143-145, 152-155, 159-163, 168-171, 178-183, 200-204, 208-214 and 218-227 (**Figure 4A**). Jameson-Wolf method of analysis showed that the antigenic index are located at positions 21-32, 50-73, 100-110, 117-130, 162-169, 175-182 and 202-228 (**Figure 4A**).

A

Pos	1 2 3 4 5 6 7 8 9 0 1 2 3 4 5	score
95	D N G V V L G A E T D F Q G S	26
212	K R N L V D V D N S F L E S K	25
79	A G G F V G G V Q A G Y N W Q	24
83	V G G V Q A G Y N W Q L D N G	24
164	A F N L G D D A S A L H T W S	22
2	K S V I L A S I A A M F A T S	21
15	T S A M A A D V V V S E P S A	21
147	R L M V Y G T G G L A Y G K V	21
163	S A F N L G D D A S A L H T W	21
203	S E Y L Y T D L G K R N L V D	21
211	G K R N L V D V D N S F L E S	21
213	R N L V D V D N S F L E S K V	21
89	G Y N W Q L D N G V V L G A E	20
61	S S F D K E D N E Q V S G S L	19
145	T E R L M V Y G T G G L A Y G	19
158	Y G K V K S A F N L G D D A S	19
58	H P F S S F D K E D N E Q V S	18
137	R A R L G Y T A T E R L M V Y	18
196	N N N W T L K S E Y L Y T D L	18
218	V D N S F L E S K V N F H T V	18

B

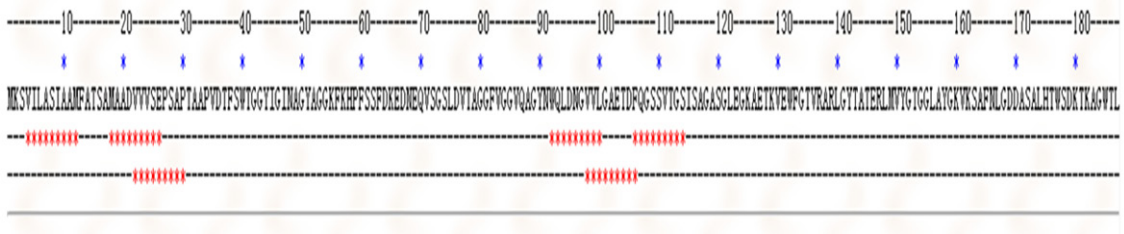

Figure 3. T-cell epitope prediction results for the OMP31 protein. Selected from the twenty highest-scoring regions with SYFPEITHI software (A). Using ProPred MHC Class-II Binding Peptide Prediction Server software (B) to predict the OMP31 protein of T-cell epitope (red represent strong specificity of position).

Using IEDB software predicted B-cell epitopes: Through IEDB online software which analyze B-cell linear epitopes of OMP31 protein, the regions with high scores are 24-40, 50-58, 61-77, 82-85, 87-89, 91-93, 104-129, 155-157, 166-174, 176-190 and 192-194 (**Figure 4B**). Through IEDB online software which analyze corner area of OMP31 protein, the regions with high scores are 108-114, 166-172, 48-54, 91-97, 104-110, 107-113, 42-48, 62-68, 25-31, 90-96, 89-95, 67-73, 40-46, 53-59, 38-44, 196-202, 68-74, 49-55, 61-67 and 118-124 (**Figure 4C**). Through IEDB online software which analyze surface accessibility of OMP31 protein, the regions with high scores are 65-70, 64-69, 177-182, 63-68, 178-183, 62-67, 142-147, 66-71, 124-129, 176-181, 179-184, 202-207, 175-180, 61-66, 200-205, 123-128, 209-214, 204-209, 26-31 and 67-72 (**Figure 4D**). Through IEDB online software which analyze the flexibility of OMP31 protein, the regions with high scores are 106-112, 64-70, 107-113, 63-69, 105-111, 65-71, 123-129, 122-128, 108-114, 121-127, 62-68, 66-72, 104-110, 109-115, 25-31, 124-130, 178-184, 177-183, 67-73 and 24-30 (**Figure 4E**). Through IEDB online software which analyze antigen index of OMP31 protein, the regions with high scores are 19-25, 22-28, 4-10, 3-9, 18-24, 21-27, 20-26, 2-8, 94-100, 71-77, 230-236, 23-29, 5-11, 98-104, 232-238, 82-88, 97-103, 17-23, 1-7 and 201-207 (**Figure 4F**). Through IEDB online software which analyze the hydrophilicity of OMP31 protein, the regions with high scores are 64-70, 66-72, 178-184, 65-71, 123-129, 67-73, 63-69, 62-68, 61-67, 68-74, 108-114, 25-31, 166-172, 103-109, 107-113, 104-110, 125-131, 208-214, 124-130 and 176-182 (**Figure 4G**).

T-and-B combined epitopes prediction results of OMP31 protein

This research use DNAStar and IEDB software for comprehensive analysis of B-cell antigen

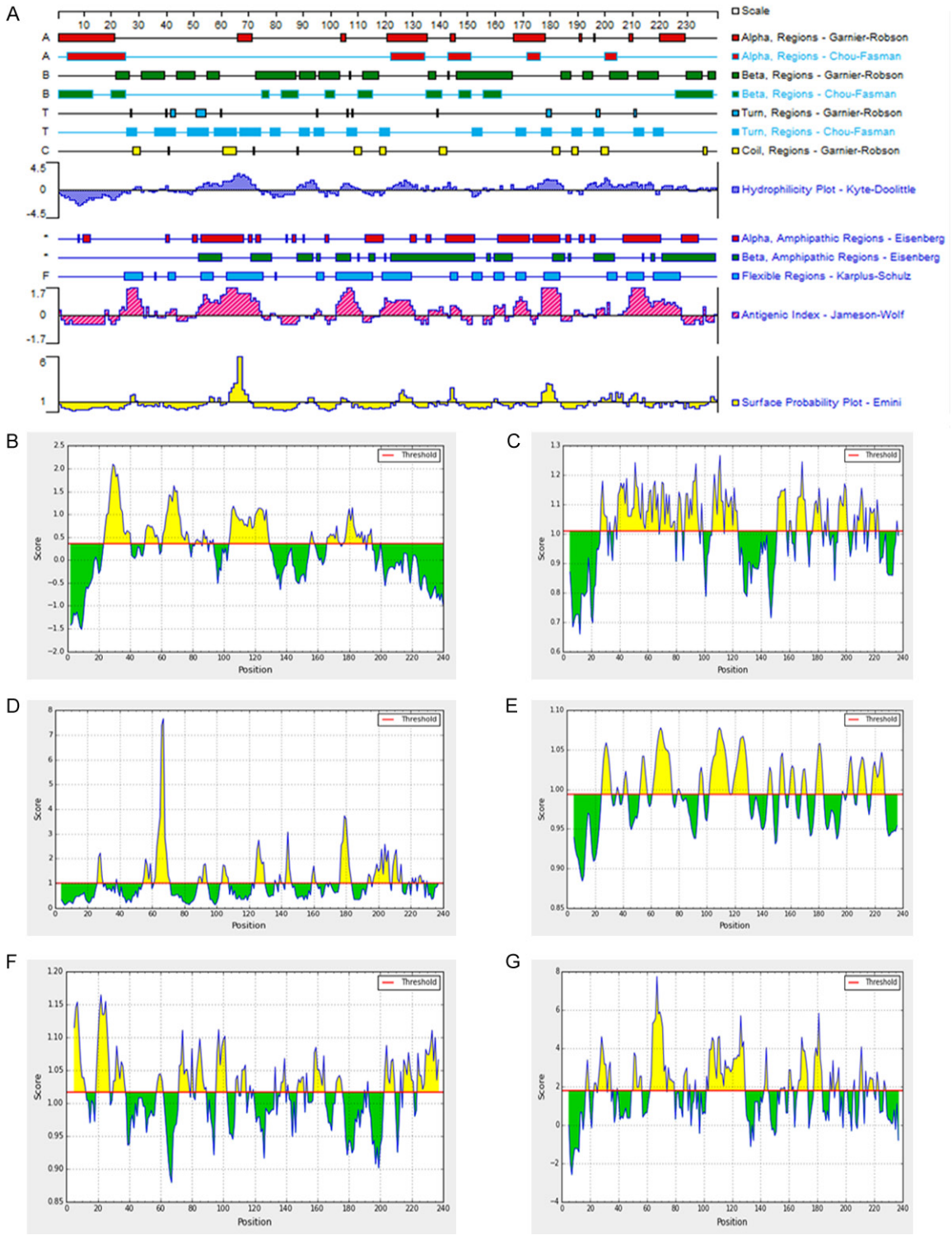

Figure 4. DNAStar Server software to predict B-cell epitope of the OMP31 protein (A). IEDB online software was used to predict the B-cell linear epitope (B), Beta-turn (C), surface accessibility (D), skeleton area flexibility (E), antigen index (F), hydrophilicity (G).

epitopes. The B-cell epitopes position of OMP31 protein are 25-31, 108-114, 201-207, 152-157, 166-172, 230-238, 123-127, 96-102 and 171-176 (**Table 1**).

Table 1. The T-and B-cell epitopes and T-B combined epitopes prediction

Epitopes	Position	Sequence
B-cell epitopes	25-31	SGPSAPT
	108-114	GSSVTGS
	201-207	LLSGTLT
	152-157	GTGGLA
	166-172	ALGAAAS
	230-238	HTVAVGLAT
	123-127	GGLAG
	96-102	AGVVLGA
	171-176	ASALHT
T-cell epitopes	19-30	AADVVVSEPSAP
	92-106	WQLDNGVVLGAETDF
	106-114	FQGSSVTGS
	137-179	RARLGYTATERLMVYGT
		GGLAYGKVKSAFNLGDD
		ASALHTWSD
	196-228	NNNWTLKSEYLYTDLGK
		RNLVDVDNSFLESKVN
T-B combined epitopes	108-114	GSSVTGS
	152-157	GTGGLA
	171-176	ASALHT

Using SYFPEITHI and ProPred MHC Class-II Binding Peptide Prediction Server software for comprehensive analysis of T-cell epitopes of OMP31 protein, T-cell epitopes are located at positions 19-30, 92-106, 106-114, 137-179 and 196-228 (**Table 1**).

According to the comprehensive analysis results of the regions with high scores and position of B-cell epitopes and T-cell epitopes of OMP31 protein, finally, we have selected the three advantages T-and-B combined epitopes of OMP31 protein: 108-114, 152-157 and 171-176 (**Table 1**).

Discussion

Brucellosis is considered as one of the great threat to public health which cannot be ignored in Xinjiang region [26, 27]. Xinjiang is one of the main pastoral areas in China, which is also a re-emerging epidemic area with a high incidence rate of brucellosis [28-30]. New effective vaccines are required to protect humans and animals against *Brucella*. The epitope-based vaccines has gradually become the hotspot of current molecular vaccine [31], that can activate B cell or T cell to protect the host against pathogens [32]. How to find epitope-based vaccines has been challenging scientists for many years [33].

In order to check whether the signal peptide would present in whole OMP31 protein, we confirmed its position is 1-19 according to S value, C value and Y value by Signal-4.1 Server. In order to improve the confidence level of the prediction results, we abandoned the signal peptide site of OMP31 protein. In order to check the transmembrane of *Brucella*, TMHMM was used to predict whether the OMP31 protein was presented on transmembrane. In this study, TMHMM predicts that all the amino acid of OMP31 protein is on the outer membrane of *Brucella*. Outer membrane amino acids provide the chance to contact to immune cells, which is suggested that the outer membrane amino acids could form epitopes more than other amino acids locations.

Antigen epitopes including T-cell epitopes and B-cell epitopes can activate the immune system by stimulating the minimum area of the antigen [34, 35]. B-cell epitopes including linear epitopes and conformational epitopes mainly induce the humoral immune response. B-cell epitopes prediction analysis is mainly consisted of alpha helix, beta Angle, surface accessibility, hydrophilicity, flexibility, antigen index and so on. Predictions of B-cell epitopes help develop vaccine and antigen-antibody interactions at the molecular level [36-38]. B-cell epitopes from DNAStar and IEDB software comprehensive analysis are 25-31, 108-114, 201-207, 152-157, 166-172, 230-238, 123-127, 96-102 and 171-176. The secondary and tertiary structures of OMP31 protein were predicted. The secondary structure of OMP31 protein prediction was performed by SOPMA Server software. We found that the proportion of random coils, and β turns accounted for 34.17% and 11.67% (**Figure 1C**) respectively. Due to the presence of hydrogen bonds which act to maintain structural stability, the helices and β sheets are very regular structures, and are not easily deformed in the secondary structure of proteins. The β turn and random coil regions are located on the surface of the protein, its structure is loose, easily to be distorted, which is conducive to ligand binding and have a high possibility of forming epitopes

[39]. On the other hand, α helices and β sheets are usually located inside the protein, which are difficult for ligand binding. Therefore, it is difficult to form the antigen epitopes. We predicted the tertiary structure of OMP31 protein and found that most parts of OMP31 protein were flexible (**Figure 2**). This flexible structure is easier to form antigen epitopes.

Cellular immunity play crucial role in the clearance of intracellular pathogens. Whether the vaccine could elicit cell mediated immune response regarded as the vital character of candidate vaccines. The immunogenic potential of candidate vaccines needs to be indicated in the context of HLA polymorphisms. Hence, our study preformed the T cell epitopes prediction taking into account MHC-II (major histocompatibility complex II) molecules [40]. $CD4^+$ T cells play a key role in controlling bacteria growth, through IFN-γ which induce $CD4^+$ T cells in the body to control Brucella infections [41]. This study predicts the epitopes which elicit HLA-II restricted $CD4^+$ T cell immune response [42, 43]. HLA-II play an important role in immune cells, they can present the specific antigen to $CD4^+$ T cells to induce the immune response, HLA-II molecules including HLA-DR, HLA-DQ, HLA-DP [44]. HLA-DR antigen encoded by HLA-DRA and HLA-DRB, and MHC-II can be used as antigen presenting or regulatory molecules participating in specific immune response and innate immune response [45]. According to the report of the frequency of HLA alleles in humans, HLA-DRB1 locus is one of the most common HLA-A alleles in Uygur of Xinjiang. Allele HLA-DRB1*0301 in the Han, Uygur and Kazak has high frequency distribution [46, 47]. This study has selected the Xinjiang people's alleles HLA-DRB1*0301 to analysis T-cell epitopes OMP31 protein by using SYFPEITHI and ProPred MHC Class-II Binding Peptide Prediction Server software and found five T-cells dominant epitopes, which respectively are 19-30, 92-106, 106-114, 137-179, 196-228 (**Table 1**).

A strong protective epitope-based vaccine should contain T- and B-combined dominant epitopes, that can induce the host to produce long-lasting immune response, in order to effectively eliminate pathogenic microorganisms [48]. Liu et al [49] found that a length of T-B combined epitopes of *echinococcus granulosus* recombinant ferritin antigen epitope by using bioinformatics software. Wang et al [50] found that Em95 protein has a good immunogenicity by predicting the *echinococcus granulosus* Em95 protein of T-B combined epitopes. Zhang et al [51] found that the recombinant protein rAg85B has a good immunogenicity by predicting T-B combined epitopes of *Mycobacterium tuberculosis* Ag85B protein, which help develop *tuberculosis* dominant epitope vaccine. In this study, based on the T-cell and B-cell epitopes information obtained, as well as the secondary and tertiary structure, using bioinformatics software we analyzed the T-B combined epitopes of OMP31 protein. The results show that there are potential T-B combined epitopes of OMP31 protein. The T-B combined epitopes were 108-114, 152-157, 171-176. These positions have proper epitopes characteristics, so could help stimulate the cellular and the humoral immunity to eliminate pathogens for construction of multivalent vaccine against *Brucella* infection.

To sum up, using the bioinformatics software, this study found out the advantage T- and B-cell epitopes and ultimately selected the four T-B combined dominant epitopes of *Brucella* OMP31 protein. This result laid foundation for constructing multivalent epitope vaccine to protect the body from *Brucella* infection.

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Disclosure of conflict of interest

None.

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