In silico Analysis of CadF epitope- based vaccine design against Campylobacter jejuni

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Abstract

Background To eradicate infectious diseases caused by microorganisms, vaccination is a popular strategy against them and can be an effective approach. Among vaccines, subunit vaccines have been used to be more effective against diseases. CadF protein of Campylobacter jejuni is one of the important antigens in the pathogenic process of the bacterium. So, the aim of this work was to do a bioinformatics study for the identification of epitope-based CadF vaccine, as a subunit vaccine. Main body CadF gene sequences were extracted from the NCBI database and suitable physic-chemical properties of CadF were evaluated by Protprom server. Some epitopes of the CadF protein with high affinity were detected by different servers, which were predicted based on MHC-peptide complex and B-cell epitopes. The results indicated that CadF is an antigenic and non-allergenic protein and provided a desirable structure for design vaccine. Among epitopes, LSDSLALRL was confirmed for the simulation of both of B and T cells. This 9-mers peptide was located in 135-143 sequences of CadF protein and interacted with HLA-A0101. The peptide isn't allergen and has the ability to be an antigen for motivating immune system. Besides, the analysis implied that the epitope structure could allow designing a vaccine against C. jejuni.

Introduction

Campylobacter jejuni (C. jejuni) is one of the significant pathogens belongs to the genus Campylobacter, which is a gram-negative, spiral, curved, and rod-shaped bacterium [1, 2]. They can be transmitted to humans through direct contact with animals, consumption of contaminated food and water, unpasteurized milk or contact with patients [1, 3]. Some gastrointestinal diseases are caused by this pathogen, which it has considered as one common bacterial cause of diarrhea, especially in children [1, 4-6]. According to researches, more than 229,000 cases of the campylobacteriosis have been reported worldwide [7]. Campylobacter jejuni can also be associated with Guillain Barre Syndrome (GBS), as a neurological disease in humans [5, 8, 9]. The disease is an autoimmune disorder which can be caused by cross-reactive immune response between LPS (Lipopolysaccharides) core of C. jejuni and gangliosides in the nerve cells of the human [10, 11]. This bacterium has been also observed in pregnant women and can cause spontaneous abortion, stillbirth, prematurity, and neonatal sepsis in many cases. Although, C. jejuni is self- limited but it is necessary to identify and confront with C. jejuni [12].

Virulence factors such as motility, adhesion, invasion, and toxin production contribute to the pathogenesis of C. jejuni [13]. Several adhesion factors e.g. CadF, CapA, JlpA, etc. are presented by C. jejuni to facilitate host–pathogen interactions [14]. Among these, CadF is one of the important proteins that bind to fibronectin (fibronectin-binding protein) and adheres the bacteria to host cell, resulting facilitate colonization. This is a 37kDa outer membrane protein has been described as a conserved and genus-specific protein. The reports have been showed that CadF can induce massive immune responses, including both humoral- and cell- immunities [7, 15].

Today, vaccination has been a very successful strategy for the eradication and prevention of the infections. Different types of vaccines are currently available, including live attenuated, inactivated or

killed, toxoid, and subunit vaccines [16, 17]. Subunit vaccines include only parts of the microorganisms, which are known as safe and effective vaccines for human and animals. Investigations have also been shown that they are capable to induce both humoral- and cell-mediated immunities against the antigens of the microorganisms. To develop an effective subunit vaccine, the identification and prediction of the antigenic epitopes by bioinformatics tools are useful [18]. Bioinformatics methods provide new theoretical approaches for the design of the vaccines based on immunological databases such as the form of epitopes, MHC alleles, molecular interactions, and docking pathogens and host cells [19-22].

Although there are some studies on the evolution of the outer membrane proteins of *C. jejuni* as vaccine candidates, CadF can be independently considered for the design of a protective vaccine[8].

Our aim was to analysis CadF protein for the identification of the epitope-based peptide candidates and to evaluate its proteomic database by In silico tools for developing a new vaccine candidate.

Method

Protein analysis and identification of conserved regions

The sequences of the CadF protein were acquired from NCBI Protein Data Bank (https://www.ncbi.nlm.nih.gov/protein) in FASTA format (accession number: AGI56319.1, AGI56318.1, AGI56317.1, AGI56316.1, AGI56315.1, AGI56314.1, AGI56313.1, AGI56312.1, CAL35585.1, AAD28174.1, and ACP52761.1). In order to evolutionary analysis, multiple alignments and phylogenetic tree of the sequences were performed using the clustalw2 tool (https://www.ebi.ac.uk/Tools/msa/clustalw2) and Molecular Evolutionary Genetics Analysis version 7 (MEGA 7) software package. Besides, the amino acid position belongs to CadF protein were also obtained by UniProt (https://www.uniprot.org(

Allergenicity and antigenicity assessment

The Allertop (www.ddg-pharmfac.net/AllerTOP) and AllergenFP (ddg-pharmfac.net/AllergenFP) web servers were used to determine the allergenicity of the protein. The Allertop server was planned based on amino acids properties such as hydrophobicity, size, and helix forming which could classify a number of allergen and non-allergen targets. The AllergenFP data based to obtain a set of options for predicting allergens. After, protective antigens of the CadF protein were forecast by Vaxijen server.

Epitope conservancy assessment

To evaluate the MHC-I and MHC-II (Major Histocompatibility Complex), epitopes were used for the IEDB (crdd.osdd.net/raghava/propred), NetCTL (www.cbs.dtu.dk/services/NetCTL), NETMHC (www.cbs.dtu.dk/services/NetMHC), NHLApred (crdd.osdd.net/raghava/nhlapred), SYFPEITHI (www.syfpeithi.de), and MHC2Pred (http://crdd.osdd.net/raghava/mhc2pred/) online servers. Each above-mentioned data bases were employed to determine scoring systems for T cell epitopes.

The B-cell epitopes were identified using the IEDB (crdd.osdd.net/raghava/propred), BCPREDS (http://ailab.ist.psu.edu/bcpred/) servers with the default setting specificity 75% and ABCpred (http://crdd.osdd.net/raghava/abcpred) server with considering threshold value 0.5. Linear and discontinuous B cell epitopes were also predicted by Bepipre server (http://www.cbs.dtu.dk/services/BepiPred). This server predicted B-cell epitopes through Hidden Markov Model (HMM) model. To select antigenicity property, each epitope gained among these servers were checked. Finally, the common and repetitive selected results were characterized and analyzed as predicted epitopes.

Design and evaluation of molecular docking

To recognize the three-dimensional structures and biological functions, Phyre2 (www.sbg.bio.ic.ac.uk/phyre2), as an online protein fold recognition server, was used. Secondary structure of the protein was also analyzed by Psipred (http://bioinf.cs.ucl.ac.uk/psipred) server.

Two TMHMM v.2.0 (http://www.cbs.dtu.dk/services/TMHMM) and Protprom (https://web.expasy.org/protparam) servers were used to predict exo-membrane amino acid sequences of CadF protein and physico-biochemical characteristics for designing a high accuracy protein vaccine. In order to gain structures of best epitopes, we have used PyMOL software (Lancaster University, UK).

Result

CadF sequences were extracted from the NCBI database and FASTA format was used for analysis. The complete sequences of CadF protein were 319 amino acids and multiple sequence alignment showed that this protein is a highly conserved protein among Campylobacters and is belonging to the superfamily of outer member proteins (ompA) and in the 193-287 position of CadF protein an ompA-like domain can be detected. The result of the phylogenic tree also confirmed that CadF is classified in outer membrane protein superfamily (data not shown).

Antigenicity and Allergenicity protein analysis

The score of the antigenic prediction was calculated 0.79 by Vaxijen server. The results showed that this protein is probably antigen and can be used for the next analysis. The obtained data from the AllergenFP server was indicated a similarity 0.82 for the protein; hence it cannot be an allergen. Allertop server analysis also confirmed that CadF protein isn't an allergen.

The physico−chemical characterizes

Using ProtParam server, the MV (molecular weight) and PI (isoelectric point) parameters were 35979.04 Da and 5.89, respectively. The aliphatic index was 69.12 and GRAVY (grand average of hydropathicity) of protein was -0.679. As a result, these amino acids of CadF protein have hydrophobicity and acidity properties (PI≤7.35). The aliphatic index was included alanine, valine, isoleucine, and leucine amino

acids, indicating thermostability of the protein. Moreover, TMHMM server analysis confirmed that CadF is an outer membrane protein.

Prediction of secondary and tertiary structures

To predict secondary and tertiary structures, we used different online servers. By PSIPRED server, graphical results of the secondary protein structures were obtained that indicated sheet, helix, and extracellular transmembrane structures (Additional file 1). In addition, The Phyre online servers were reported a three-dimensional structure of the modeled CadF with 97% confidence score and 192 known-domains aligns. The structural contents are included 16% alpha helix, 41% beta strands, and 16% disordered regions. Also, the prediction of the CadF protein shows a binding site at GIU-HIS-LYS residue and a lot of metallic heterogenic sections in its structure (Fig. 1). Moreover, the three-dimensional (3D) structure belongs to selected epitopes was drawn by PyMOL software (Fig. 2).

Forecasted of antigenic T cell epitopes

To predict T cell epitopes, the best score of the epitopes were selected from SYFPEITH, IEDB, NetCTL, NHLAPred, NETMHC I, and MHCPred II online servers. Except by IEDB, which showed a high value for the lowest number, other servers have been specified a high value for the highest number. The epitopes of the MHC I (A-0101, A-0201, and B-2705) and the MHC II (DR1-0101 and DRB1-0401) were the most common epitopes in Iranian alleles that have been considered in this study. According to achieved data from above-mentioned servers, the predicted epitopes of MHC I and MHC II are presented in Tables 1 and 2, respectively.

Among selected epitopes, LLCLGLASV, RRVDAKFIL, FSADNNVKF, and LSDSLALRL (belong to MHC class I) are shown in Table 1 and EGHFGFDKTTINPTF, QINFNHANH, LSDSLALRL, ASVLFSADNNVKFEI, and QINFNHANHNWVSTL (belong to MHC II) are showed in Table 2, as well. Due to achieving a high score in many servers and being antigen and loss of allergenicity, we estimated that they can act as a proper epitope.

B-cell epitopes prediction

After extracting the B-cell epitopes by IEDB, Bcepred, ABCpred, and SVMTrip servers, common and repetitive epitopes were selected. The results are showed in Table 3. According to data, WVSTLGISFG, LETRDQINFN, VGEKFYFYGL, and NPRSSNDTKEGRADNRRVDA peptides were characterized that could be analyzed as predicted B-cell epitopes. Also, the graph was modeled by Bepipred server and in Fig. 3 shows that the yellow parts can be B-cell epitopes with a suitable threshold (0.5). Y-axe shows scores related to the amino acids and X-axe defines positions related to the protein regions.

Overall result of above-mentioned epitopes

The retrieved results of tables have identified the favored residues from T- and B-cells called LSDSLALRL, because it is common epitope with antigenicity and allergenicity properties and we suggest it as a

candidate vaccine for next analysis.

Discussion

Campylobacter jejuni is one of the main reasons for gastroenteritis diseases throughout the world, specifically in developing countries[3, 6]. It can be associated with diarrhea (from mild to severe), fever, neurological disorders, reactive arthritis, and weight loss. It is estimated about 14 cases for 100,000 people that were afflicted worldwide every year [4, 23]. In order to handle the disease, the sciences are in urgent to raising their knowledge about C. *jejuni*. The pathogenicity of *C. jejuni* is up to the ability to linkage with other organisms and many proteins take part in the adhesion and colonization of bacteria[24]. Among adhesive proteins of the bacteria, many studies approved that the CadF protein could consider as a proper candid for vaccination projects. In this study, we focused on the immunogenic protein of the CadF to design vaccine through bioinformatics tools which can dramatically reduce the number of In vitro tests [20].

In recent years, the bioinformatics knowledge provides easy accession to get information around the design of genomics, proteomics, and new vaccine of the bacteria. With regard to development of recombinant vaccines, predicting this type of vaccines has advantages rather than other methods. In silico vaccines can be considered as time-saving and convenient methods to identify antigens, allergens, and reducing the laboratory errors. In addition, they are cheaper than other traditional methods; while the anticipation of the epitope regions doesn't always carry out with 100% accuracy and the results of several servers don't probably present the same results [19, 21, 25, 26].

The past studies have been declared some efforts to suggest an effective vaccine against *C. jejuni.* For instance, some sciences have used the whole-cell vaccines of the *C. jejuni* and it had advanced in animal models but has failed at the human cases. Since the results were not able to gotten approved in term of safety for humans [5]. Also, different reports have demonstrated polysaccharide capsule (cps) of the bacteria that could be used for alleviating campylobacteriosis, but this approach was just tested at phase 1 clinical; however there are concerns about being the similarity between bacterial polysaccharides and human gangliosides [10].

Other antigens, *e.g.* ABC transporter (PEB1), which is known as an immunogenic and protective protein, could be a candidate for vaccination against *C. jejuni*. Due to the response of the immune system produced by lymphocytes inverse PEB1 protein, it could inhibit the development of the disease. However, this vaccine has been building in pre-clinical trials yet [5, 27]. Beyond a PEB1-vaccine mentioned approach, other pathogenic proteins of *C. jejuni* have also been analyzed. Kobierecka et al. have found that CjaA, CjaD (binding proteins), FlaA (flagellin), and CmeC (outer-membrane protein) could protect the chickens against *C. jejuni* in many cases [9]. Neal-McKinney et al. also have asserted the vaccination of chickens with the CadF-FlaA-FlpA fusion protein that could reduce the rate of infection, but there is not any evidence whatever will be able to use in human models [8]. Moreover, some of the peptides related to invasive, virulent, and membrane of the bacteria, which contain FlaA, Cia, CadF, PEB1, PEB3, and MOMP

have assessed which could be effective in design a vaccine against *C.jejuni* by immunoinformatics tools. So, despite many efforts to make the vaccine, there are not any approved the vaccine against *C. jejuni* to be useful in humans so far [7, 18, 19].

Our findings showed that CadF is a highly conserved protein among *Campylobacter* spp. and is belonging to the superfamily of outer member proteins (ompA). [28-30].

We collected T-cell and B-cell epitope regions through different servers and the higher epitopes have elicited to make an effective vaccine against *C. jejuni.* The final common certain epitopes are included LLCLGLASV, RRVDAKFIL, FSADNNVKF, LSDSLALRL, EGHFGFDKTTINPTF, QINFNHANH, ASVLFSADNNVKFEI, QINFNHANHNWVSTL, WVSTLGISFG, LETRDQINFN, VGEKFYFYGL, and NPRSSNDTKEGRADNRRVDA. These epitopes were confirmed to act as humoral- and cell-mediators and induced an immune response and a motivate immune system according to Vaxijen and Allertop data.

In addition, our research also showed the correct topology model based on phyre2 server that predicts CadF is a stable target. This analysis was done with bioinformatics methods and helps to design novel vaccine according to sequence profile and spatial structure and dimension of protein.

In present study used from SYFPEITH, IEDB, NetCTL, NHLAPred, NETMHC I, MHCPred II, Bcepred, ABCpred, and SVMTrip online servers for searching of our study. Finally, common epitopes were identified and the LSDSLALRL epitope was selected as the best potential vaccine candidate. Moreover, the allergenicity of the LSDSLALRL epitope has also confirmed by Allertop server and confirmed that this sequence wasn't an allergen as well.

The epitope was located in 135-143 regions and can be interacted with HLA-A0101 according to collected results from many above-mentioned servers. In a partial contrary by Yasmin study, who gained its knowledge based on just IEDB and SYFPEITHI servers on CadF protein, suggested that FRLSDSLAL epitope from the protein can be as good chosen for deigning vaccine [19].

It is clear that our epitope has fairly matched with the epitope presented by Yasmin et al. (77.77% of amino acids are matched, <u>LSDSLAL</u>RL and FR<u>LSDSLAL</u>, which are marked by underline) and this similarity can be a higher claim for designing an effective vaccine against *C. jejuni*. Based on Allertop server, the presented epitope by Yasmin et al. was an allergen, while no allergenicity was observed for our epitope "LSDSLALRL" in this study.

In addition, CadF is a significant protein for the colonization and binding the bacteria to host cells and maximum connection can be detected in the regions of the fibronectin-binding domain including phenylalanine-arginine-leucine-serine (FRLS) residues of CadF. Although only fifty percent from the amino acids of our epitope was identified as the binding site to host cells, multiple servers confirmed that this reign has a high score for developing the vaccine.

According to aliphatic index, alanine, valine, isoleucine, and leucine amino acids were detected in structure of the protein, which proposed it as a thermostable protein. These amino acids in thermophilic

bacteria, such as *C. jejuni*, are significantly higher than that of ordinary proteins [31]. This is another advantage of CadF that can be proposed it for the development of the vaccine. Heat stability is an important feature in vaccine production that can simplify the logistics of vaccine distribution and expand the immunization coverage [32]. So, it has been supporting from the most crucial role of this sequence of CadF protein at disease trend and being useful analysis to construct the active vaccine.

Conclusion

Our investigate shows bioinformatic databases play a considerable role in vaccine development and identify immunity through followed reaction CadF protein with B- and T- cells In silico procedures. Therefore, we suggest CadF protein of *C. jejuni* can be used to prepare an effective vaccine to prevent the disease at future analysis. However, in order to predict an actual vaccine without any side effect, we need to improve our knowledge of the pathogenesis and molecular structure of *C. jejuni* on both in vivo and in vitro studies in association with in silico researches

Abbreviations

CPS: polysaccharide capsule

ompA :outer member proteins

CadF: Campylobacter adhesion to Fibronectin

Fn: fibronectin

MHC: Major Histocompatibility Complex

Declarations

Limitations

Limitation in the use of the some servers.

Authors' Contributions

MMN, SS, MMN, and BB involved in the management of the project, the analysis of data, and writing up the paper. All authors read and approved the final manuscript.

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Conflict of interest

No conflict of interest.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

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Tables

Table 1. The list of high scored predicted T cell epitopes using online software and their Vaxijen and Allertop score. The purple highlighted epitopes were repeated in some servers but aren't selected because of being problems in their antigenicity and allergenicity. The blue- colored epitopes are suitable with regard to their traits and the green epitope was considering the most common peptide with correct abilities.

				MHC **I**	
equence	Allele	Server	Score	Vaxigen score	AllerTOP Score
EKFY	HLA-A01	SYFPEITHI	26	0.017 (Probable NON-ANTIGEN)	NON-Allergen
SNAAY			26	0.39 (Probable NON-ANTIGEN)	NON-Allergen
LEHY			25	1.2 (Probable ANTIGEN)	Allergen
YNY			22	1.2 (Probable ANTIGEN)	Allergen
RTY			21	0.5 (Probable ANTIGEN)	Allergen
ASV	HLA02:01		32	0.43 (Probable ANTIGEN)	NON-Allergen
FSA			23	0.33 (Probable NON-ANTIGEN)	NON-Allergen
ONNV			23	0.05 (Probable NON-ANTIGEN)	NON-Allergen
TDNI			23	0.05 (Probable ANTIGEN)	Allergen
GIRL	HLA-B2705		26	1.68 (Probable ANTIGEN)	NON-Allergen
AKFIL			26	1.45 (Probable ANTIGEN)	NON-Allergen
OSLAL			24	1.35 (Probable ANTIGEN)	Allergen
INFNH			24	0.34 (Probable NON-ANTIGEN)	Allergen
SAIK			23	0.27 (Probable NON-ANTIGEN)	Allergen
LASV	HLA-A*02:01	IEDB	0.3	0.30 (Probable NON-ANTIGEN)	NON-Allergen
GSRA	HLA-A*01:01		0.4	1.49 (Probable ANTIGEN)	Allergen
KFIL	HLA-B*27:05		0.4	1.45 (Probable ANTIGEN)	NON-Allergen
LFGA	HLA-A*02:01		0.5	0.19 (Probable NON-ANTIGEN)	NON-Allergen
NAAY	HLA-A*01:01		0.55	0.39 (Probable NON-ANTIGEN)	NON-Allergen
NNVKF		NetCTL	1.3198	0.66 (Probable ANTIGEN)	NON-Allergen
YNY	HLA-A0101		2.376	1.2 (Probable ANTIGEN)	Allergen
LEHY			1.380	1.2 (Probable ANTIGEN)	Allergen
RTY			1.8915	0.56 (Probable ANTIGEN)	Allergen
RTYL			1.4347	0.13 (Probable NON-ANTIGEN)	NON-Allergen
FSNAAY			1.7677	0.39 (Probable NON-ANTIGEN)	NON-Allergen
SLALRL			2.0179	1.82 (Probable ANTIGEN)	NON-Allergen

Table 2. The selected MHC II class binding epitopes were summarized according to most score predicted by several servers and assess their antigenicity and allergenicity. The purple highlighted epitopes were repeated in some servers but aren't selected because of being problems in their antigenicity and allergenicity. The blue- colored epitopes are suitable with regard to their traits and the green epitope was considering the most common peptide with correct abilities.

| MHC II | | | | | |
Sequence	Allele	Server	Score	Vaxigen score	Allertop Score
GHFGFDKTTINPTF	HLA-DRB1*04:01	IEDB	1.70	0.42 (Probable ANTIGEN)	NON-Allergen
EGHFGFDKTTINPT			1.74	0.2 (Probable NON-ANTIGEN)	NON-Allergen
HFGFDKTTINPTFQ			1.76	0.31 (Probable NON-ANTIGEN)	NON-Allergen
LASVLFGADNNVKF			1.77	0.47 (Probable ANTIGEN)	NON-Allergen
ASVLFGADNNVKFE			1.77	0.67 (Probable ANTIGEN)	Allergen
SVLFGADNNVKFEI			1.77	0.71 (Probable ANTIGEN)	Allergen
FGFDKTTIN	HLA-DRB1*0101	MHC2Pred	1.517	0.33 (Probable NON-ANTIGEN)	Allergen
QINFNHANH			1.415	1.08 (Probable ANTIGEN)	NON-Allergen
LSAIKGID			1.248	0.065 (Probable NON-ANTIGEN)	Allergen
GRADNRRVD			1.157	2.78 (Probable ANTIGEN)	NON-Allergen
YNQKLSERR	HLA-DRB1*0101		1.117	1.60 (Probable ANTIGEN)	NON-Allergen
PQAKCPVEP			0.901	0.053 (Probable NON-ANTIGEN)	Allergen
KVLDENERY			0.786	0.15 (Probable NON-ANTIGEN)	Allergen
NLDMDNRY			0.785	0.23 (Probable NON-ANTIGEN)	Allergen
TYLSAIKGIDVGEK	HLA DRB10101	SYFPEITHI	30	0.13 (Probable NON-ANTIGEN)	NON-Allergen
FYFYGLAGGGYEDF			28	0.72 (Probable ANTIGEN)	NON-Allergen
GVKFRLSDSLALRLE			27	2.11 (Probable ANTIGEN)	Allergen
NHNWVSTLGISFGFG			27	0.86 (Probable ANTIGEN)	Allergen
LDMDNRYAPGIRLG			26	1.30 (Probable ANTIGEN)	Allergen
QINFNHANHNWVSTL	HLA DRB1-0401		28	0.63 (Probable ANTIGEN)	NON-Allergen
EGHFGFDKTTINPTF			28	0.42 (Probable NON-ANTIGEN)	NON-Allergen
LEKYGVEKSRIKTVG			28	0.50 (Probable ANTIGEN)	NON-Allergen
SVLFSADNNVKFEI			26	0.58 (Probable ANTIGEN)	NON-Allergen

Sequence				Vaxijen score	Allertop
NNVKFEITPTLNYN			26	1.36 (Probable ANTIGEN)	Allergen
LSDSLALRL	HLA-A0101	NETMCH	0.15	1.82 (Probable ANTIGEN)	NON-Allergen
HTDNIGSRA			0.17	1.49 (Probable ANTIGEN)	Allergen
YEDFSNAAY			0.40	0.39 (Probable NON-ANTIGEN)	NON-Allergen

Table 3. The list of high scored predicted B cell epitopes using online software and their Vaxijen and Allertop scores. The purple highlighted epitopes were repeated in some servers but aren't selected because of being problems in their antigenicity and allergenicity. The blue- colored epitopes are suitable with regard to their traits and the green epitope was considering the most common peptide with correct abilities.

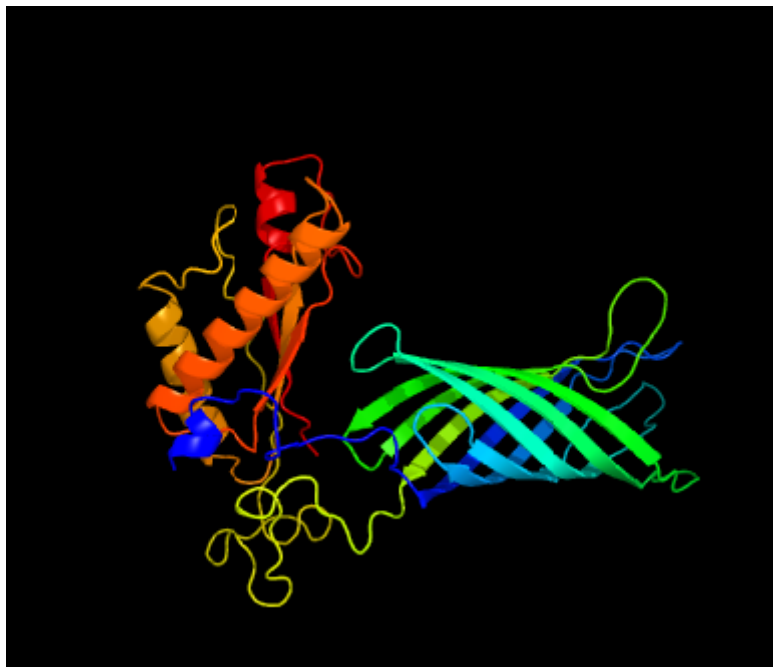| sition Sequence	Server	Score	Vaxigen score	Allertop Score
IFLCLGLASVLFG	IEDB	13	0.36 (Probable NON-ANTIGEN)	NON-Allergen
APGVRLGYHFDD		12	0.81 (Probable ANTIGEN)	NON-Allergen
3FSNAAYDNKS	ABCpred	0.81	0.29 (Probable NON-ANTIGEN)	NON-Allergen
NNVKFEITPT		0.79	1.33 (Probable ANTIGEN)	Allergen
9WVSTLGISFG		0.79	0.62 (Probable ANTIGEN)	NON-Allergen
8YDNKSGGFGH		0.78	0.79 (Probable ANTIGEN)	NON-Allergen
4LETRDQINFN		0.76	0.89 (Probable ANTIGEN)	NON-Allergen
VGEKFYFYGL		0.75	0.52 (Probable ANTIGEN)	Allergen
2SGGFGHYGAG		0.75	0.82 (Probable ANTIGEN)	NON-Allergen
0GGKKEKAVEEVADTRPAPQA	BCPREDS	0.3	0.30 (Probable NON-ANTIGEN)	Allergen
5NPRSSNDTKEGRADNRRVDA		0.4	1.49 (Probable ANTIGEN)	NON-Allergen
4GHFGFDKTTINPTFQEKIKE		0.4	1.45 (Probable ANTIGEN)	NON-Allergen
SDVKYTNTNKTTDITRTYLS		0.5	0.19 (Probable NON-ANTIGEN)	NON-Allergen
GIDVGEKFYFYGLAGGGYED		0.55	0.39 (Probable NON-ANTIGEN)	NON-Allergen
5NAAYDNKSGGFGHYGAGVKF		0.55	0.39 (Probable NON-ANTIGEN)	Allergen
4LETRDQINFNHANHNWVSTL		0.55	0.39 (Probable NON-ANTIGEN)	Allergen
DMDNRYAPGVRLGYHFDDFW		0.55	0.39 (Probable NON-ANTIGEN)	NON-Allergen
7VLDENERYDTILEGHTDNIG		0.55	0.39 (Probable NON-ANTIGEN)	NON-Allergen
FGADNNVKFEITPTLNYNYF		0.55	0.39 (Probable NON-ANTIGEN)	Allergen
9NDTKEGRADNRRVDAKFILR	SVMTrip	1.000	2.04 (Probable ANTIGEN)	Allergen
0HTDNIGSRAYNQKLSERRAK		0.822	1.43 (Probable ANTIGEN)	NON-Allergen
KKIFLCLGLASVLFGADNNV		0.548	0.1 (Probable NON-ANTIGEN)	NON-Allergen

Additional File

Figures

Figure 1

Three-dimension structure of CadF protein was predicted. The image colored by rainbow from N to C terminus. The Alpha helix (yellow and orange) and beta strand (green and blue arrows) and linker (thin strands).
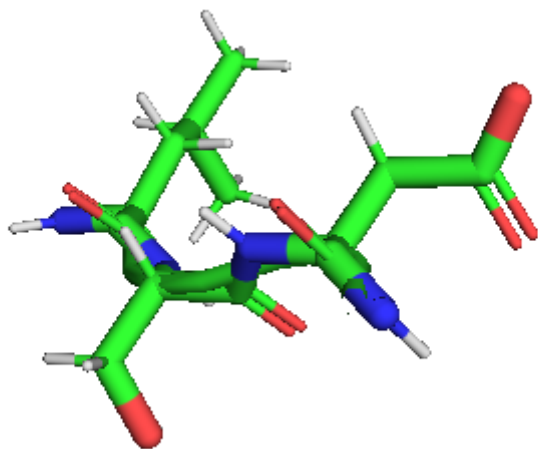

Figure 2

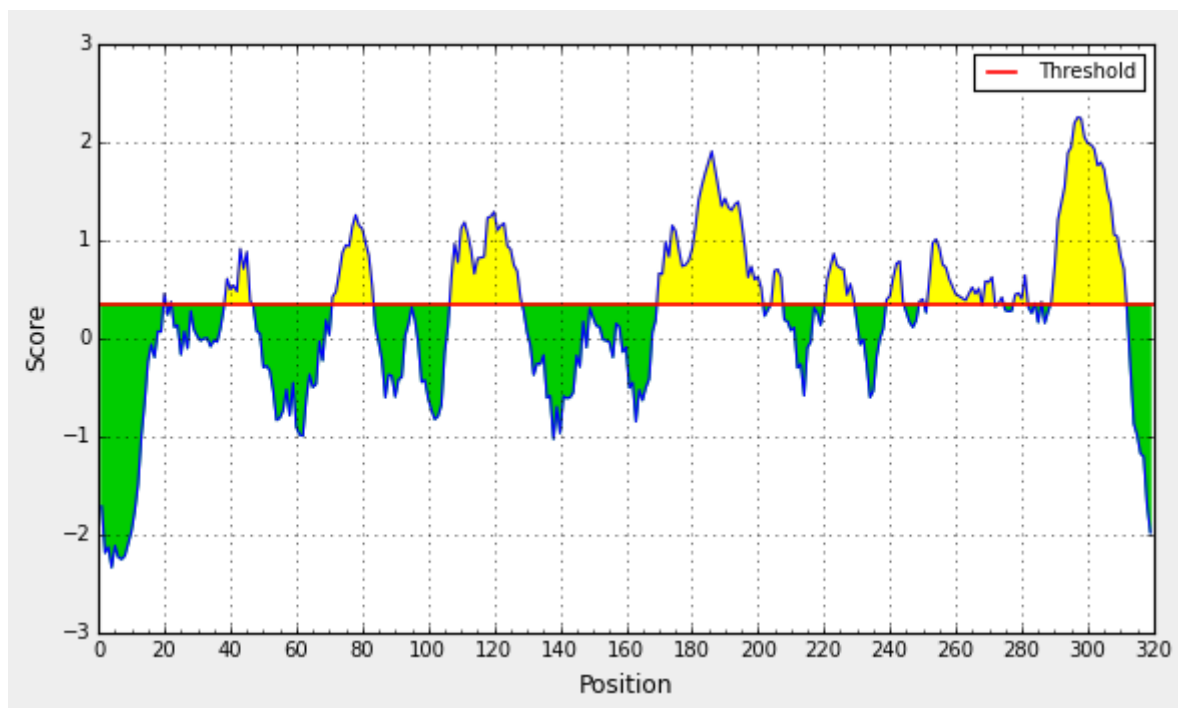the structure of predicted epitope" LSDSLALRL" was painted by Pymol software.

Figure 3

Prediction B cell epitopes from Length proteins by IEDB. The amino acids may be able to epitopes were shown in yellow regions above the threshold line .there are a range of epitopes at the 160 -200 and 250 to 315 range approximately.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementary1.pdf