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Prediction of Conformational and Linear B-Cell Epitopes on Envelop Protein of Zika Virus Using Immunoinformatics Approach

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Abstract

The current spread of Zika virus infection in India has become a public health issue due to the virus's possible link to birth abnormalities and neurological disorders. There is a need for enhanced vaccines or drugs as a result of its epidemic outbreak and the lack of potential medication. B-cell mediated adaptive immunity is capable of developing pathogen-specific memory that confers immunological protection. Therefore, in this study, the envelope protein of the Zika virus was retrieved from the NCBI protein database. The ABCpred and BepiPred software were used to discover linear B-cell epitopes on envelope protein. Conformational B-cell epitopes on envelope protein were identified using SEPPA 3.0 and Ellipro tools. Predicted B-cell epitopes were evaluated for allergenicity, toxicity, and antigenicity. Two consensus linear B-cell epitopes, envelope$_{165-180}$ (AKVEITPNSPRAEATL) and envelope$_{224-238}$ (PWHAGADTGTPHWNN) were identified using ABCpred and BepiPredtools. SEPPA 3.0 and Elliprotools predicted consensus conformational envelope$_{98-110}$ (DRGWGNGCGLFGK) and envelope$_{248-251}$ (AHAK) epitopes and one residue (^{75}PRO) within envelope protein as a component of B-cell epitopes. These predicted linear and conformational B-cell epitopes will help in designing peptide vaccines that will activate the humoral response. However, in-vitro and in-vivo laboratory experimental confirmations are still needed to prove the application's feasibility.

Keywords Immunoinformatics · Envelop protein · B-cell epitopes · Vaccine · Zika virus

Introduction

The Zika virus is a newly emerging virus that belongs to the Flaviviridae family of viruses (Hamel et al. 2015). In recent years, Zika virus infections have caught the interest of the national and international medical community, owing to their role in causing microcephaly and other neuro-developmental disorders as a result of maternal infections (Kuno et al. 1998). The recent outbreaks of the Zika virus in India are a cause of great concern. In late 2018, 159 instances of Zika virus infection were recorded in Rajasthan (Yadav et al. 2019) and 127 cases in Madhya Pradesh (Saxena et al. 2019). Since most Zika virus infections are moderate or asymptomatic, the magnitude of these outbreaks is likely underestimated, and the consequences might be serious if the trend continues into 2019. Maharashtra reported its first laboratory-confirmed Zika infection on July 31, 2021, from Belsar village in the Pune district (WHO, 2021). On July 8, 2021, a citizen of Kerala state, southwest India, was confirmed to be infected with the Zika virus (WHO, 2021). There are currently no effective antiviral medications available for Zika virus infection (Saiz and Martín-Acebes 2017). Berberine, which has previously been used to treat the dengue virus, also had a significant affinity for the Zika virus NS3 protease (Sahoo et al. 2016).

A humoral immune response to the Zika virus is produced by ZIKV-specific B cells, which result in ZIKV-specific plasma cells that secrete IgG and IgA. During the initial infection, IgM is formed, followed by the production of high affinity antibodies with significant opsonizing capabilities against ZIKV structural proteins (Priyamvada et al. 2017; Lai et al. 2018). Antibodies produced in response to ZIKV infections against EDIII are potent neutralizing antibodies that can give protective immunity (Robbiani et al. 2017). Antibodies produced against ZIKV non-structural proteins,

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pre-membrane, and EDI/II show little or no neutralizing ability and are typically cross-reactive antibodies.

Despite numerous types of research on Zika antiviral therapies, no FDA-approved category of medicine has been determined to be safe for use in pregnant women (Munjal et al. 2017). Antigen-antibody interaction is important in immunological processes and reactions within an immune system, and the regions of antigens that are identified and bound by B-cell produced antibodies are known as B-cell epitopes (Van Regenmortel 1989). In addition, B-cell epitopes can be used to activate the humoral immune response in the presence of certain cross-reacting antibodies (Walter 1986; Van Regenmortel 2004). Approach of fast distinguishing proof of immuno epitopes is focused on computational prediction, which use advance calculations and expanding epitope information base. Epitope expectation is one of the main foundations of in-silico immunization planning, but it relies upon antigen ID, and most vitally epitope determination for a powerful resistant reaction. The upside of computational vaccinology is usage of high through put information investigation strategies for quick antigen ID, molecular docking and reproduction models to test immunological reactions. This strategy can examine numerous antigen up-and-comers and entire proteomes for antigenicity and viability in a generally brief time frame. As a result, identifying B-cell epitopes has become a major component of epitope-based vaccine design. B-cell epitopes are classified into two types: linear epitopes and conformational epitopes. Linear epitopes are composed of residues that are continuous in the sequence, whereas conformational epitopes are composed of residues that are distantly separated in the sequence yet have spatial closeness. The wet experiment for epitope identification takes a long time, is labor-intensive, and is costly. With the increasing availability of experimentally produced epitopes, it is feasible to build computer approaches for epitope prediction that are both faster and less expensive (Flower 2007). We used bioinformatics approaches to study the envelope protein of the Zika virus and predict its structure. On this basis, we predicted the linear and conformational B cell epitopes, which provided a theoretical basis for the vaccine development and prevention of the Zika virus.

Materials and Methods

ZIKV Envelope Protein Sequence

The amino acid sequence of the ZIKV envelope protein were extracted from the polyprotein sequence (GenBank ID: AZS35409.1) that is deposited in the NCBI protein database. The Vaxign version 2.0 beta server (Xiang and He 2009), a vaccine target prediction and analysis tool based on reverse vaccinology, was utilized to analyze envelope protein as a potential vaccine target for designing vaccine candidates.

Envelope Protein Sequence Analysis

The antigenicity of the envelope protein sequence was determined using the VaxiJen v2.0 server (https://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) (Doytchinova and Flower 2007). The Protparam software was used to predict the physicochemical properties of the envelope protein, such as the instability index, grand average of hydropathicity (GRAVY), theoretical pI, and molecular weight (Walker 2005; Wilkins et al. 1999). The protein's allergenicity was determined using AllerTOP v.2.0 (Dimitrov et al. 2014).

Prediction of Linear B-Cell Epitopes

ABCpred (Saha and Raghava 2006) and BepiPred 2.0 (Larsen et al. 2006) were used to predict linear B cell epitopes. Consensus predicted Linear B cell epitopes from both methods were chosen. Using the VaxiJen v2.0 server, the antigenicity of the epitopes was identified. The server AllerTOP v.2.0 was used to compute the allergenicity of epitopes (Dimitrov et al. 2014). AllerTOPv.2.0 is a web-based allergenicity prediction tool that produces accurate findings independent of alignment. Furthermore, using an SVM-based algorithm, ToxinPred was used with default parameters to predict the toxicity of epitopes (Gupta et al. 2013).

Homology Modeling of The Envelope Protein

The homology model of envelope protein was built using the SWISS-MODEL server (Waterhouse et al. 2018). The Swiss-Model server's Structure Assessment page (https://swissmodel.expasy.org/assess) was used to validate the model's quality using Ramachandran plots. Another method used to validate the model structure was ProSA-web (Wiederstein and Sippl 2007). If the Z-score (total model quality score) falls outside of the expected range for native proteins, the structure is likely to be incorrect.

Prediction of Conformational B Cell Epitopes

SEPPA 3.0 (Zhou et al. 2019) and Ellipro (Ponomarenko et al. 2008) were utilized in predicting conformational B-cell epitopes based on the 3D structure of a protein. Conformational B-cell epitopes that were predicted by both methods were chosen as final.

Results

Vaccine Target

The envelope protein was investigated as a possible vaccine target using the Vaxign version 2.0 beta server (Xiang and He 2009). Two transmembrane helixes were predicted in envelope protein. The calculated adhesion probability for this protein is 0.713. If the probability of adhesion is greater than 0.51, the protein is an adhesion. The predicted protein has no similarity to human proteins.

Envelope Protein Sequence Analysis

The protein antigenicity score of 0.6268 indicates an excellent vaccine antigenic feature. The protein had a molecular weight of 54410.22 Da and was non-allergenic. The protein has a negative GRAVY value (− 0.087), indicating that the protein was hydrophilic and had a high interaction with water molecules. The envelope protein has an instability value of 22.77, indicating that it is a stable protein. The protein's theoretical pI is 6.51, indicating that it is acidic. The AllerTOP v.2.0 tool indicates that protein is a non-allergen.

Linear B-Cell Epitopes Analysis

Two types of bioinformatics tools searched for the full-length sequence of envelope protein for probable sequential B-cell epitopes. The ABCpred server discovered a total of 28 epitopes with a threshold of 0.80, as shown in Table 1. The BepiPred 2.0 tool predicted 11 optimal B-cell epitopes at a threshold of 0.35, as shown in Table 2. Overlapped epitopes were presented as bold in Tables 1 and 2. Antigenicity, toxicity and allergenicity of identified B-cell epitopes were shown in Tables 1 and 2. Epitopes with antigenic, non-toxic, and non-allergenic properties that were often predicted by the ABCpred and BepiPred 2.0 tools were chosen. On this basis,

Table 1 Identified B-cell epitopes on envelope protein using ABCpred tool.

Sl. No.	Start position	ABCPRED predicted B cell epitope	ABCPRED Score	Antigenicity	Antigen/ non- antigen	Toxicity	Allergenicity
1	327	TVEVQYAGTDGPCKVP	0.94	0.2878	Non- Antigen	Non-Toxin	Allergen
2	165	**AKVEITPNSPRAEATL**	0.94	0.8044	Antigen	Non-Toxin	Non-allergen
3	183	FGSLGLDCEPRTGLDF	0.91	1.6185	Antigen	Non-Toxin	Allergen
4	156	TGHETDENRAKVEITP	0.90	1.0173	Antigen	Non-Toxin	Non-allergen
5	149	SGMIVNDTGHETDENR	0.89	0.2236	Non- Antigen	Non-Toxin	Non-allergen
6	32	TVMAQDKPTVDIELVT	0.88	0.6246	Antigen	Non-Toxin	Non-allergen
7	216	EWFHDIPLPWHAGADT	0.88	0.3163	Non- Antigen	Non-Toxin	Allergen
8	61	YEASISDMASDSRCPT	0.87	0.4350	Antigen	Non-Toxin	Non-allergen
9	305	YSLCTAAFTFTKIPAE	0.87	0.1812	Non- Antigen	Non-Toxin	Non-allergen
10	118	KFACSKKMTGKSIQPE	0.87	0.3581	Non- Antigen	Non-Toxin	Non-allergen
11	282	GRLSSGHLKCRLKMDK	0.86	0.4552	Antigen	Non-Toxin	Allergen
12	224	**PWHAGADTGTPHWNNK**	0.86	0.8358	Antigen	Non-Toxin	Non-allergen
13	17	GGTWVDVVLEHGGCVT	0.86	0.3396	Non- Antigen	Non-Toxin	Allergen
14	99	RGWGNGCGLFGKGSLV	0.85	0.1925	Non- Antigen	Non-Toxin	Allergen
15	422	AVLGDT**AWDFGSV**GGA	0.85	1.4526	Antigen	Non-Toxin	Non-allergen
16	392	GEKKITHHWHRSGSTI	0.84	0.2253	Non- Antigen	Non-Toxin	Allergen
17	356	GRLITANPVITESTEN	0.84	0.1273	Non- Antigen	Non-Toxin	Allergen
18	464	SQILIGTLLMWLGLNT	0.83	0.3328	Non- Antigen	Non-Toxin	Non-allergen
19	43	IELVTTTVSNMAEVRS	0.83	0.8137	Antigen	Non-Toxin	Non-allergen
20	362	NPVITESTENSKMMLE	0.83	0.4414	Antigen	Non-Toxin	Non-allergen
21	197	DFSDLYYLTMNNKHWL	0.83	1.1716	Antigen	Non-Toxin	Allergen
22	416	RGAKRMAVLGDTAWDF	0.82	1.0717	Antigen	Non-Toxin	Non-allergen
23	5	GVSNRDFVEGMSGGTW	0.81	0.5890	Antigen	Non-Toxin	Non-allergen
24	128	KSIQPENLEYRIMLSV	0.81	1.0989	Antigen	Non-Toxin	Non-allergen
25	78	GEAYLDKQSDTQYVCK	0.80	0.1017	Non- Antigen	Non-Toxin	Non-allergen
26	69	ASDSRCPTQGEAYLDK	0.80	0.2907	Non- Antigen	Non-Toxin	Allergen
27	374	MMLELDPPFGDSYIVI	0.80	0.7070	Antigen	Non-Toxin	Allergen
28	333	AGTDGPCKVPAQMAVD	0.80	− 0.0114	Non- Antigen	Non-Toxin	Allergen

These are the cases selected for further evaluation

Table 2 Identified B-cell epitopes on envelope protein using Bepipred 2.0 tool.

Sl. No.	Start	End	Bepipred predicted B cell epitope	Antigenicity	Antigen/ Non- Antigen	Toxicity	Allergenicity
1	12	18	VEGMSGG	0.2209	Non- Antigen	Non-Toxin	Non-allergen
2	36	41	QDKPTV	0.1977	Non- Antigen	Non-Toxin	Non-allergen
3	66	89	SDMASDSRCPTQGEAYLDKQSDTQ	0.3833	Non- Antigen	Non-Toxin	Allergen
4	125	134	MTGKSIQPEN	1.2685	Antigen	Non-Toxin	Allergen
5	155	181	DTGHETDENR**AKVEITPNSPRAEATL**G	0.7359	Antigen	Non-Toxin	Non-allergen
6	189	196	DCEPRTGL	0.2428	Non- Antigen	Non-Toxin	Allergen
7	224	238	**PWHAGADTGTPHWNN**	0.5401	Antigen	Non-Toxin	Non-allergen
8	274	284	EAEMDGTKGRL	0.6060	Antigen	Non-Toxin	Allergen
9	331	342	QYAGTDGPCKVP	− 0.2179	Non- Antigen	Non-Toxin	Allergen
10	364	371	VITESTEN	0.3222	Non- Antigen	Non-Toxin	Non-allergen
11	428	434	**AWDFGSV**	2.0657	Antigen	Non-Toxin	Non-allergen

These are the cases selected for further evaluation

two B-Cell epitopes envelope$_{165-180}$ (AKVEITPNSPRAEATL) and envelope$_{224-238}$ (PWHAGADTGTPHWNN) were identified.

Modeling and Tertiary Structure Validation of Model Protein

Homology modeling of the target protein's 3D structure was done in stages, commencing with a template structure search on the Swiss-Model server. From a large number of hits, a template structure (PDB-Id: 6CO8) was picked as the model's construction. The target sequence has 100% query coverage, and the sequence identity with the template sequence is 99.80%. Based on the template and target alignment, the Swiss-Model server built a homology model of the target sequence.

The Swiss model/Structure assessment page was utilized to validate the tertiary structure of the model using Ramachandran plot analysis. Figure 1a shows a Ramachandran plot of the predicted model with 92.59% of residues in the favored region and 0.40% in the outlier region. The ProSA-web server calculates the total quality score for a given input structure and displays it in the context of all known protein structures. Using the ProSA web server, the

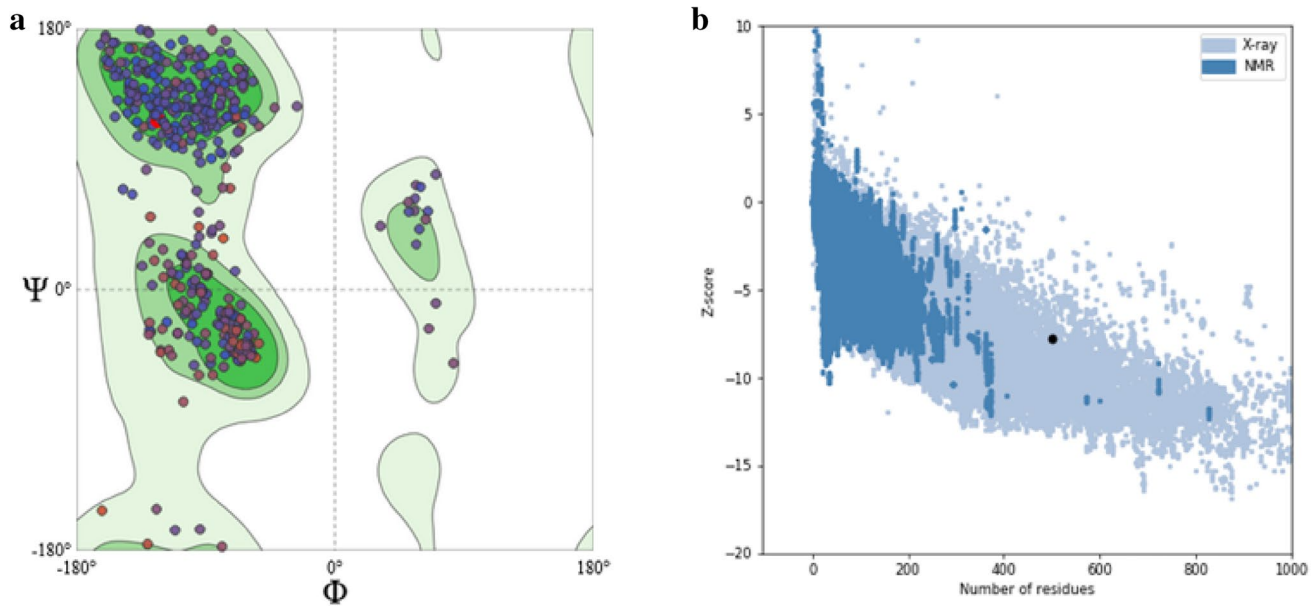

Fig. 1 The Ramachandran plot and the ProSA-web server were used to validate the tertiary structure of the model protein. **a** Ramachandran plot shows that the number of amino acid residues in the favorable region is 92.59%. **b** The ProSA-web result gives a Z score of − 7.78.

model protein Z score was −7.78, as shown in Fig. 1b, in broad black dot.

Conformational B-Cell Epitopes Analysis

Conformational B-cell epitopes on envelope protein were identified by SEPPA 3.0 (Zhou et al. 2019) at a threshold of 0.17 as shown in Fig. 2.3D representations of the predicted discontinuous residues on envelope protein by SEPPA 3.0 were shown in Fig. 2a and predicted residues in Fig. 2b.

Ellipro (Ponomarenko et al. 2008) at a threshold of 0.9 predicted conformational B-cell epitopes, were shown in Fig. 3. 3D representations of the predicted discontinuous residues on envelope protein were shown in Fig. 3a & c. Predicted residues having scores of 0.962 and 0.936 by ElliPro were shown in Fig. 3b & d.

B-cell epitope scanning and analysis using both SEPPA 3.0 and Ellipro tools predicted two potent conformational B-cell epitopes, envelope$_{98-110}$ (DRGWGNGCGLFGK) and envelope$_{248-251}$ (AHAK), as well as one residue within envelope protein (^{75}PRO) involved in discontinuous epitope formation, as shown in Fig. 4.

Discussion

ZIKV infection is a major cause of morbidity and mortality around the world. Unfortunately, the lack of effective ZIKV vaccines has resulted in the loss of many innocent lives in various parts of the world. As a result, there is an urgent need for preventive measures to combat the global threat of a ZIKV outbreak. Researchers in many countries have quickly used immunoinformatics methods to anticipate probable antigenic epitopes on the ZIKV envelope protein

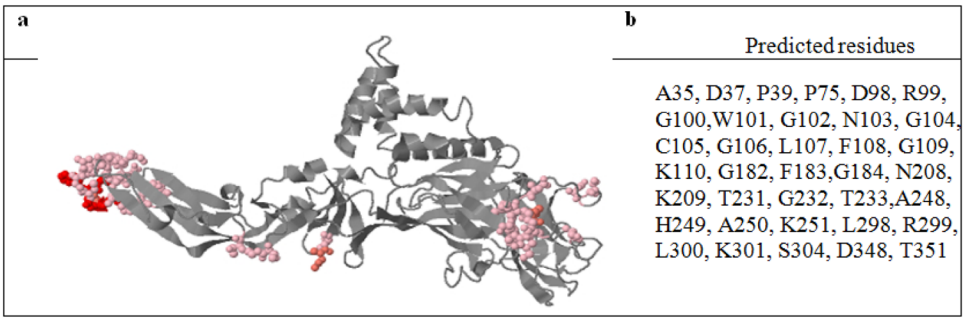

Fig. 2 Identified conformational B-cell epitopes by SEPPA 3.0. **a** 3D representation of the predicted discontinuous residues on envelope protein was shown as balls. Color of balls was red when score > = 0.2 and salmon when score > = 0.17. **b** Predicted residues.

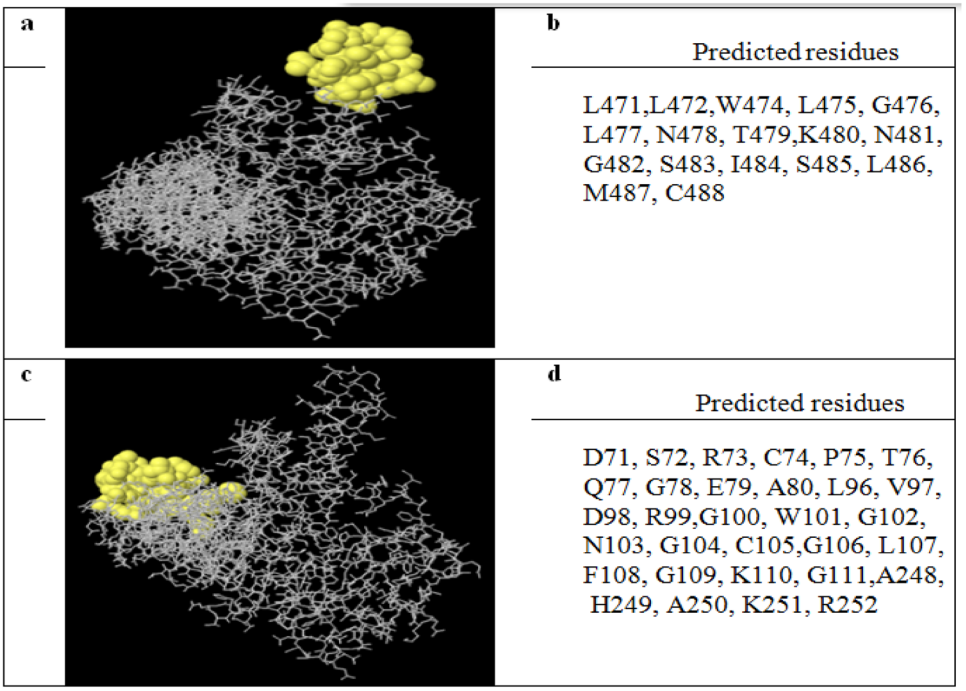

Fig. 3 Identified conformational B-cell epitopes by ElliPro server. **a** 3D representation of the predicted discontinuous residues on envelope protein was shown as a spacefill model with a score 0.962. **b** Predicted residues have a score 0.962 by ElliPro. **c** 3D representation of the predicted discontinuous residues on envelope protein was shown as a spacefill model having score of 0.936. **d** Predicted residues having a score 0.936 by ElliPro server.

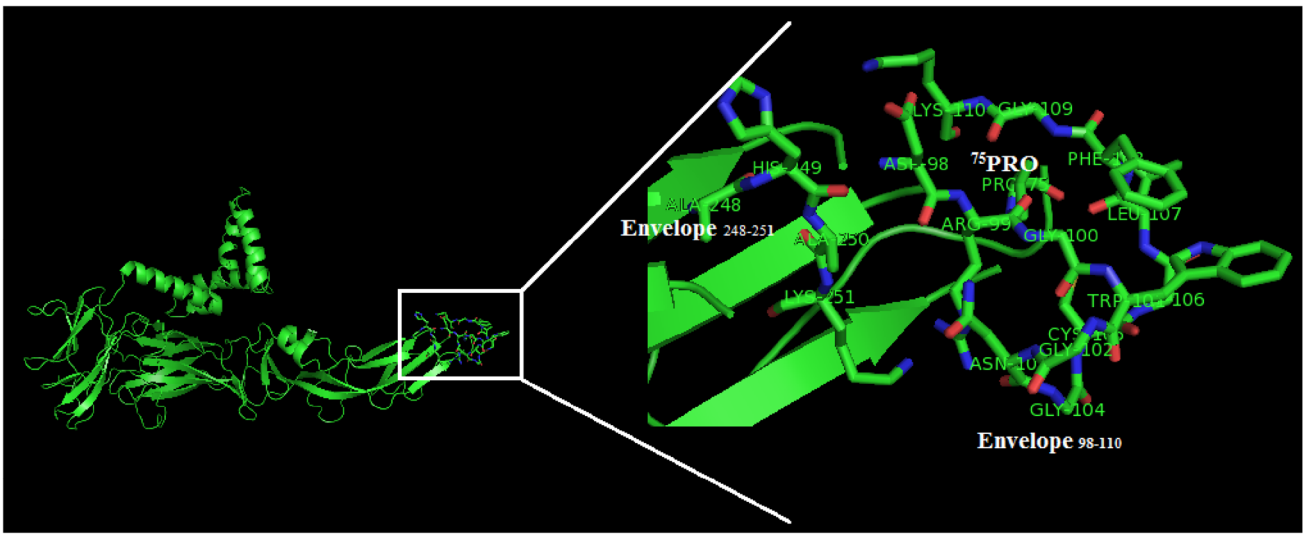

Fig. 4 The consensus predicted conformational B-cell epitopes and residues involved in discontinuous epitope formation on envelope protein by both SEPPA 3.0 and Ellipro tools.

for peptide vaccine production (Ashfaq and Ahmed 2016; Dikhit et al. 2016; Dar et al. 2016; Weltman 2016; Gupta 2014; Badawi et al. 2016). Pandey et al. demonstrated the use of a combinatorial immunoinformatics method to produce a multi-epitope subunit vaccine using Zika virus structural and nonstructural proteins (Pandey et al. 2018). In the current study, we have predicted linear as well as conformational B-cell epitopes on envelope protein using immunoinformatics tools. In recent years, several researchers have employed immunoinformatics methods to develop epitope-based vaccines for Dengue virus (Krishnan G et al. 2020), Orthohantavirus (Joshi et al. 2022), and SARS-CoV-2 (Sarkar et al. 2020; Rahman et al. 2020). By targeting sandfly saliva and parasite-derived membrane and secretory antigens, Ojha et al. developed a multi-epitope vaccination for *Visceral leishmaniasis* (Ojha et al. 2020).They predicted immunogenic B-cell, HTL, and CTL epitopes from all of the identified protein sequences. By utilizing *Leishmania donovani* membrane proteins, Khatoon et al. developed a multi-epitope subunit vaccine using a combinatorial strategy (Khatoon et al. 2019). To create the subunit vaccine, cytotoxic T- and helper T-lymphocyte binding epitopes were sequentially combined with appropriate adjuvant and linkers. ABCpred and BepiPred tools predicted two consensus linear B-cell epitopes, envelope$_{165-180}$ (AKVEITPNSPRAEATL) and envelope$_{224-238}$ (PWHAGADTGTPHWNN), which had a good antigenicity score, non-toxic and non-allergenic. Different researchers utilized similar tools to identify sequential B-cell epitopes on SARS-CoV-2 spike protein (Chen et al. 2020). The Swiss-Model server created a homology model of the envelope protein sequence based on the template structure (PDB-Id: 6CO8). Results of the Ramachandran plot and the ProSA-Web server indicate that the overall model quality is good. Envelope$_{98-110}$ (DRGWGNGCGLFGK) and Envelope$_{248-251}$ (AHAK) were discovered as consensus epitopes, as well as one envelope protein residue (^{75}PRO) as a conformational B-cell epitope component, via the SEPPA 3.0 and Ellipro servers. These predicted epitopes were antigenic, non-allergenic, and non-toxic. On the SARS-CoV-2 envelope protein, one conformational B-cell epitope was predicted using similar tools, SEPPA 3.0 and Ellipro (Lon et al. 2020). B-cell epitopes are predicted to stimulate a humoral immune response, which should be verified in both in vivo and in vitro models. Antigenic B-cell epitopes predicted and analyzed in this research work might present a preliminary set of peptides for future vaccine development against ZIKV.

Conclusion

Epitope search enjoys an extra benefit to additional limited down the antigen evaluating for extremely short unambiguous regions, subsequently giving a chance where protein-based control can be utilized to cooperative energies and select the proper resistant reaction type. This research identifies new potential B-cell epitopes for fighting Zika virus infections and may lead to the development of ZIKV vaccines. Because the findings of this study are based on computational methodologies, laboratory experiments in-vitro and in-vivo are required to validate this work.

Author Contributions All authors reviewed the manuscript

Declarations

Conflict of interest The author declares that there is conflict of interest to disclose.

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