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IN SILICO DESIGN OF B-CELL EPITOPE BASED PEPTIDE VACCINE FOR ZIKA VIRUS

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ABSTRACT

Zika virus infection attracted the attention of the medical community since it is transmitted by the Aedes mosquito and humans act as hosts. The disease affects fetal development and causes severe neurodevelopmental disorders, such as GBS (Guillain-Barre Syndrome), and CZS (Congenital Zika Syndrome) in pregnant women, including congenital microcephaly, and fetal death. Therefore, a vaccine is needed for prevention. Epitope-based peptide vaccines have advantages in terms of both selectivity and safety. The use of computational methods is a cost-efficient way of developing vaccines. This research aims to look at conserved areas and see the phylogenetic tree of the zika virus E protein sequences obtained from various countries, to see the most immunogenic epitope notifications of the ZIKV E protein sequence using the in-silico method, to see the potential for the most immunogenic epitopes of protein sequences. Zika virus as a vaccine candidate through the use of in silico. This study was using a descriptive observational study using in-silico tools for Zika virus peptide vaccine candidates. Some software and websites that were used are MEGA-X, IEDB, VaxiJen 2.0, BLASTp NCBI. From the 41 sequences that have been collected, 3 epitope candidates had antigenic properties and also passed the similarity test so the potential to develop a peptide vaccine; SLGLDCE, ETDENRAKVEVTPNSPRAEATLG, and AHAKRQ.

Keywords:*Epitope, In Silico, Peptide,Vaccine, Zika Virus*

ABSTRAK

Infeksi virus Zika belakangan ini menarik perhatian kalangan medis karena dapat ditularkan oleh nyamuk Aedes dan manusia berperan sebagai inang. Penyakit yang ditimbulkan berdampak pada perkembangan janin dan dapat menyebabkan kelainan perkembangan saraf yang parah, seperti GBS (Guillain-Barre Syndrome), CZS (Congenital Zika Syndrome) pada wanita hamil termasuk mikrosefali kongenital, bahkan kematian janin. Oleh karena itu, diperlukan vaksin sebagai bentuk preventif. Vaksin peptida berbasis epitop memiliki kelebihan baik dari segi selektivitas dan keamanan. Penggunaan metode komputasi merupakan cara tepat dengan efisiensi biaya untuk pengembangan vaksin. Penelitian ini bertujuan untuk mencari kandidat epitop dari protein E virus Zika yang memiliki potensi sebagai vaksin peptida melalui tinjauan in silico. Penelitian ini termasuk penelitian observasional deskriptif secara in silico menggunakan beberapa software dan website yakni MEGA-X, IEDB, VaxiJen 2.0, BLASTp NCBI. Dari 41 sequence yang diperoleh, terdapat 3 kandidat epitope yang memiliki sifat antigenik dan juga lolos dari uji similaritas sehingga berpotensi dilakukan pengembangan vaksin peptida, yaitu ; SLGLDCE, ETDENRAKVEVTPNSPRAEATLG, dan AHAKRQ.

Kata kunci: *Epitop,In Silico,Peptida, Vaksin, Virus Zika,*

INTRODUCTION

Zika virus (ZIKV) is a member of the Flaviviridae family and is closely related to Dengue Virus (DENV), West Nile Virus (WNV), Japanese Encephalitis Virus (JEV), Yellow Fever Virus (YFV). ZIKV was first reported and isolated from sentinel rhesus monkeys in Uganda in 1947. ZIKV is transmitted by the Aedes mosquito and humans act as hosts[1]. In addition to mosquito bites, Zika virus transmission can also be transmitted through sexual intercourse, the mother to the fetus, and blood transfusions [2]. Zika virus genetic material is a positive sense, single-stranded ribonucleic acid (RNA) virus. RNA is translated into a single polyprotein that encodes structural proteins, namely the Capsid (C), Membrane (M), and Envelope (E). The non-structural proteins include NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 [3]. The ZIKV E protein is the primary target of the humoral response. Efficient antibodies will exhibit strong binding characteristics and block the E protein region that mediates important functions as a host cell entry site [4].

In 2013 there was a Zika virus epidemic in French Polynesia [1], [5]. In 2019, exactly in July, it was recorded that 87 countries and regions had evidence of transmission of the Zika virus (ZIKV) transmitted by mosquitoes, spread in four of the six WHO regions, namely Africa, America, Southeast Asia, and the Western Pacific [6]. In Indonesia, the first reported human infection was in 1977 in Central Java [1]. In Asian countries, cases of congenital malformations associated with ZIKV, microcephalus, and fetal death have been identified. Based on epidemiological data from WHO, globally 61 countries and regions have evidence of competent Aedes aegypti vectors but have not recorded ZIKV transmission. Therefore, there is still a potential risk of ZIKV spreading to other countries [6].

Zika virus infection has recently attracted the attention of the medical community. The resulting disease does not have a high mortality rate, but it does have an impact on fetal development and can cause severe neurodevelopmental abnormalities [7]. Mild symptoms of infection include fever, rash, conjunctivitis, headache, malaise, muscle aches, and pain . In some cases, Zika Virus infection can cause severe illness, such as GBS (Guillain-Barre Syndrome) in adults. Zika virus infection also affects CZS (Congenital Zika Syndrome) in pregnant women including congenital microcephaly, and even fetal death in infected women during pregnancy [2], [8]. In addition, people infected with ZIKV may experience eye disorders ranging from mild conjunctivitis to severe chorioretinal lesions [9]. Currently, there is no specific treatment and vaccine for Zika virus prevention, the only treatment for clinical symptoms arises through pharmacological and non-pharmacological treatment.

The Aedes mosquito is found all over the world, so transmission through mosquito vectors will likely spread widely. Therefore, vaccines are needed as a form of prevention. In March 2016, WHO reported 18 ZIKV vaccine programs, and currently, the ZIKV vaccine is still in the development process with various formulations and methods being studied, including live virus vaccines, inactivated virus vaccines, and whole virus vaccines, subunit vaccines, and vaccines. messenger RNA (mRNA), DNA, protein, and vector-based formulations [10], [11]

Several previous researchers also searched for vaccine designs and tried to find the best candidates for T and B cell epitopes using an immunoinformatics approach (Prasasty et al., 2019). Meanwhile, Adianingsih [12]identified a conserved B cell epitope of glycoprotein protein on the ZIKV envelope through an intensive in silico study. However, the data obtained need to be re-validated using antigenicity analysis as the author will do.

Vaccine development can be facilitated by using immunoinformatics and computational methods. In addition, the use of computational methods is an appropriate way with cost efficiency for the development of simulations and calculations in drug design. This method is very relevant for epitope-based vaccine design because it has advantages, namely in terms of selectivity and safety [9], [13] Therefore, the development of vaccine candidates will be carried out using the help of viral epitopes through protein sequences to identify the most immunogenic part of the virus. The advantage of protein vaccines is that the approach is more focused on precisely finding the epitope region on the antigen and presenting it for the immune response. It started with collecting data in the form of Zika virus E protein sequence at NCBI, then analyzing the data using MEGA-X software, The Immune Epitope Database (IEDB), VaxiJen 2.0, Basic local alignment search tool protein (BLASTp).

MATERIAL AND METHOD

Zika Virus Sequence Collection

The author used the ZIKV E protein amino acid sequence database from the NCBI website (https://www.ncbi.nlm.nih.gov/labs/v)

Analysis of Phylogenetic Tree and Conserved Regions with Mega X Application

The sequences obtained were then analyzed using the Mega X Application to find the Phylogenetic Tree (No. of Boostrap Replication=1000). Bootstrap values of 100 to 1000 times of replication are used to estimate the confidence level of a phylogenetic tree[14]. The next step is to perform sequence alignment using the Align by ClustalW method to find a conserved region.

Epitope mapping with the IEDB website

Epitope Analysis using the IEDB website (http://tools.iedb.org/bcell/) aims to find candidate epitopes that will be used as candidate vaccines. Several methods can be used so that they can be adjusted to the needs. IEDB Website Analysis with Bepipred Method The bepipred method is an analysis used to search for candidate epitopes that target linear B cells. Bepipred uses a threshold of 0.350 which is the default threshold from the IEDB. This method has been used in many studies using the Hidden Markov Model as an algorithm [15]. The Kolaskar and Tongaonkar antigenicity scales based on amino acid residues of physicochemical properties and frequency of tendencies known as epitope experiments have an accuracy of 75% [16]. Kolaskar and Tangaonkar methods to determine the antigenic area using a threshold of 1.024. Emini method with a threshold of 1,000[17]. This threshold is used to predict the probability of surface exposure of amino acids in selected sequences. This value is the default value and is widely used in similar studies.

Antigenicity Analysis Using Vaxijen 2.0

VaxiJen 2.0 is a reliable and consistent website for antigen prediction which does not need protein alignment in its working process, this website recognizes antigens based on the main chemical properties of the amino acid sequence via Wold's Z-scale, which converts the derived string into a uniform vector with auto cross-covariance (ACC) to describe the hydrophobicity of amino acids, their molecular size, and polarity. This test will produce data in the form of the words "probable antigen" or "probable non-antigen" accompanied by an antigenicity score that has been analyzed using a threshold of 0.5 and this threshold is considered to have the highest accuracy[18].

Epitope Protein Similarity Analysis with Human Surface Proteins with BLASTp

Analysis using BLASTp (https://blast.ncbi.nlm.nih.gov/Blast.cgi ?PAGE=Proteins) aims to determine the degree of similarity between antigenic proteins and surface proteins in the human body, which in this case are B cells and T cells. The acceptable value so that the epitope can be used is below 70% [12].

RESULT AND DISCUSSION

Sequence collection obtained from NCBI and as many as 41 sequences of Zika virus E protein originating from 6 countries, namely: Brazil 8 sequences, French Polynesia as many as 14 sequences, Fiji Islands 5 sequences, Samoa 8 sequences, Singapore 3 sequences, and the USA as many as 3 sequences. Vaccine design candidates that will be made are constructed from conserved areas from the sequences that have been obtained.

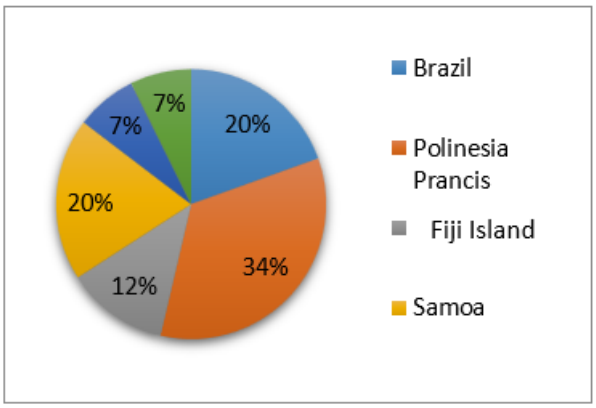

Fig 1. Zika Virus Protein Sequence Diagram by Country of Origin

Sequence Analysis Using the MEGA-X App

MEGA-X software is not only a sequence alignment but can construct a phylogenetic tree. The construction results of 41 Zika virus Protein E sequences are as follows:

Fig 1. Phylogenetic Tree from ZIKV E Protein

The phylogenetic tree above consists of several branches, that French Polynesia, Samoa, Brazil, Fiji Islands, USA, and Singapore have kinship relationships, both close and distant. Therefore, research related to this still needs to be developed to better know the phylogenetic of the Zika virus globally.

Genetic studies have revealed that the Zika virus evolved into 3 distinct genotypes: West African, East African, and Asian. This virus originated in East Africa and then spread to West Africa and Asia≈50–100 years ago [19]. In (Figure 2) the Zika virus strains in the USA, Samoa, Singapore, Brazil, the Fiji Islands, and French Polynesia are related and are included in the Asian genotype. The Zika virus has evolved gradually and spread geographically throughout Asia and the Pacific. As is the case with all RNA genomic viruses, ZIKV mutates rapidly due to its highly error-prone RNA-dependent-RNA-polymerase nature. The high mutation rate of RNA viruses enhances the ability of these viruses to adapt to diverse hosts and cause novel diseases in humans and animals [20].

Zika virus strains need to be determined in sequence parts that do not experience mutations, namely conserved regions. Performed alignment with this MEGA-X application aims to make it easier to identify sequences originating from various countries so that they can determine conserved regions. From the downloaded sequence, there are 3 conserved regions with potential antigens, including:

Table 1 . Conserved Region of ZIKV E Protein

No.	Conserved Region Sequence
1	IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMA EVRSYCYEASISDMASDSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNGCGLF GKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTG
2	ETDENRAKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWL VHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAV HTALAGALEAEMDGAKGKL
3	SGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCK IPVQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKI

Having conserved regions as a template is important in vaccine design, which has an impact on the effectiveness of the vaccine so that it can be applied globally because the sequences were found in various Zika viruses studied. The existence of genetic diversity is a major challenge in vaccine design against viral strains, so it is necessary to design vaccines to focus on introducing immunity to these conserved areas, which can be a viable strategy to increase the effectiveness of vaccines against various strains [21].

B Cell Epitope Analysis Using the IEDB Website

As for analyzing B cell epitopes from the conserved region, various methods have been obtained, including the Kolaskar & Tangaonkar, Bepipred, and Emini methods. This method is used to predict specific areas in proteins that bind to B cell receptors, these areas must be on the surface and are immunogenic [12]. Following are the results of mapping B cell epitope with various methods. Epitope mapping results are displayed in graphic form, the yellow area is considered to have high potential for antigenicity. Values equal to or greater than the threshold value are said to have a strong potential to bind to B cells. The following graph shows the potential for epitope of B cells.

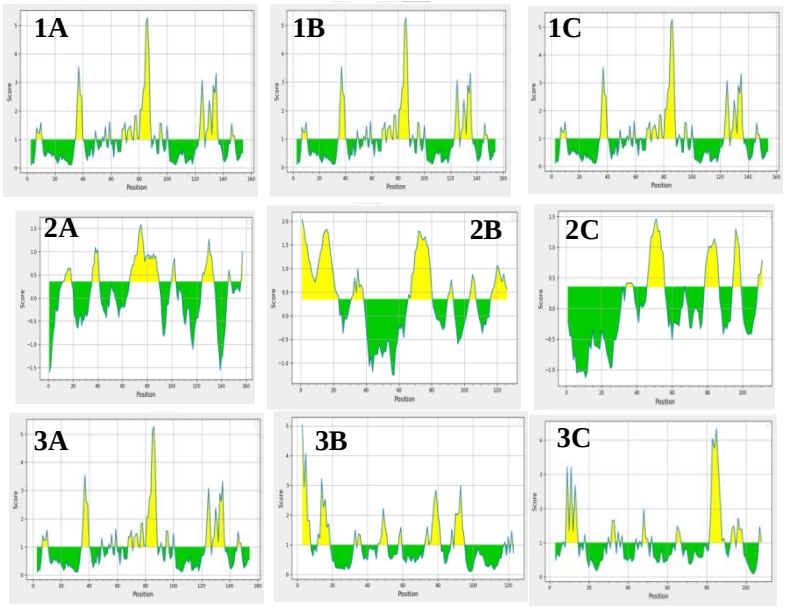

Fig 3. Graphic of B cell Epitope Mapping using Kolaskar and Tangaonkar methods; (1A) Conserved Region 1, (1B) Conserved Region 2, (1C) Conserved Region 3. Graphic of B cell Epitope Mapping using the Bepipred method; (2A) Conserved Region 1, (2B) Conserved Region 2, (2C) Conserved Region 3. Graphic of B cell Epitope Mapping using the Emini method; (3A) Conserved Region 1, (3B) Conserved Region 2, (3C) Conserved Region 3

Note : Yellow color indicates sequences that have epitope potential, while green color indicates sequences that do not have epitope potential.

The important thing to note regarding the epitope to be developed into a vaccine is the surface accessibility of the predicted epitope because it will interact with antibodies to get an immune response. Then, hydrophilicity and flexibility are important characteristics of epitopes in immunogens which should also be considered with the highest priority to obtain a better immune response [15].

VaxiJen 2.0 Website Antigenicity Potential Analysis

VaxiJen 2.0 website used to validate the results of the epitope sequences found on the previous IEDB website whether immunogenic or not.This check will produce statistics within the shape of the words "possibly antigen" or "probably non-antigen" observed by an antigenicity score that has been analyzed the use of a threshold of 0.5[18].

Table 2. VaxiJen 2.0 Analysis of B-Cell Epitope Mapping by Kolaskar & Tangaonkar, Bepipred, and Emini Methods

Threshold	IEDB Method	Epitope Peptide Prediction	Result	Antigenicity Score
0.5	Kolaskar & Tangaonkar	WVDVVLEHGGCVTVM	Non Antigen	0.3924
0.5	Kolaskar & Tangaonkar	DIELVTTT	Probable Antigen	1.6414
0.5	Kolaskar & Tangaonkar	VRSYCYEA	Probable Antigen	0.6724
0.5	Kolaskar & Tangaonkar	TQYVCKRT	Non Antigen	0.1147
0.5	Kolaskar & Tangaonkar	KGSLVTCAKFACS	Non Antigen	0.3472
0.5	Kolaskar & Tangaonkar	MLSVHGS	Non Antigen	0.0326
0.5	Kolaskar & Tangaonkar	SLGLDCE	Probable Antigen	1.7129
0.5	Kolaskar & Tangaonkar	FSDLYYL	Probable Antigen	0.7406
0.5	Kolaskar & Tangaonkar	WLVHKE	Non Antigen	0.1613

0.5	Kolaskar & Tangaonkar	DIPLPWH	Probable Antigen	0.9158
0.5	Kolaskar & Tangaonkar	RQTVVVLGS	Probable Antigen	0.8102
0.5	Kolaskar & Tangaonkar	GAVHTALAGA	Non Antigen	0.2221
0.5	Kolaskar & Tangaonkar	LKGVSYSLCTAA	Non Antigen	0.3797
0.5	Kolaskar & Tangaonkar	HGTVTVEVQYA	Probable Antigen	1.2198
0.5	Kolaskar & Tangaonkar	PCKIPVQMA	Probable Antigen	0.5921
0.5	Kolaskar & Tangaonkar	LTPVGRL	Probable Antigen	1.1528
0.5	Kolaskar & Tangaonkar	DSYIVIGV	Probable Antigen	1.1964
0.5	Bepipred	VEGMSGG	Non Antigen	0.2209
0.5	Bepipred	QDKPTV	Non Antigen	0.1977
0.5	Bepipred	SDMASDSRCPTQGEAY LDKQSDTQ	Non Antigen	0.3833
0.5	Bepipred	MTGKSIQPEN	Probable Antigen	1.2685
0.5	Bepipred	ETDENRAKVEVTPNSP RAEATLG	Probable Antigen	0.6092
0.5	Bepipred	DCEPRTGL	Non Antigen	0.2428
0.5	Bepipred	PWHAGADTGTPHWNN	Probable Antigen	0.5401
0.5	Bepipred	QYAGTDGPCK	Non Antigen	-0.1106
0.5	Bepipred	VITESTEN	Non Antigen	0.3222
0.5	Emini	AQDKPT	Non Antigen	0.2663
0.5	Emini	QGEAYLDKQSDT	Non Antigen	0.1168
0.5	Emini	KSIQPENLE	Probable Antigen	1.5482
0.5	Emini	TPNSPRA	Non Antigen	-0.4616
0.5	Emini	LTMNNK	Probable Antigen	2.5695
0.5	Emini	TGTPHWNNK	Probable Antigen	1.6563
0.5	Emini	AHAKRQ	Probable Antigen	1.1540
0.5	Emini	KMDKLR	Non Antigen	-2.7336
0.5	Emini	ESTENSKM	Probable Antigen	0.7702

From the results of the study using the VaxiJen 2.0 website, the highest score from the Kolaskar & Tangaonkar method was sequence 2 with the epitope "SLGLDCE" having a score of 1.7129. The highest antigenicity score for the Bepipred method was 1.2685 which was found in sequence 1 with the epitope “MTGKSIQPEN”. The highest score on the Emini method is 2.5695 on “LTMNNK”. However, a high antigenicity score cannot necessarily be directly proposed as a vaccine candidate, because it is necessary to test for similarity analysis.

Similarity Analysis Using NCBI's BLASTp Website

Similarity analysis using BLASTp aims to see the similarity between the VaxiJen 2.0 validated epitope and surface receptors in the human body so that it can prevent the occurrence of autoimmune where the immune system can attack the body itself[22]. 14 candidate epitopes will be tested for similarity using BLASTp as follows. It needs to be passed 70% to be accepted and considered to not have similarities with surface cell proteins.

Table 3. Results of Epitope Similarity Test

Epitope Peptide Prediction	Antigenicity Score	BlastP Result
DIELVTTT	1.6414	Have similarities with cell surface proteins
SLGLDCE	1.7129	Has nothing in common with cell surface proteins of human
HGTVTVEVQYA	1.2198	Has nothing in common with cell surface proteins of human
LTPVGRL	1.1528	Have similarities with cell surface proteins (kinase protein)
SDMASDSRCPTQGEAYL DKQSDTQ	0.3833	Has nothing in common with cell surface proteins of human
MTGKSIQPEN	1.2685	Have similarities with cell surface proteins (kinase protein)

ETDENRAKVEV TPNSPRAEATLG	0.6092	Has nothing in common with cell surface proteins of human
PWHAGADTGTPHWNN	0.5401	Has nothing in common with cell surface proteins of human
KSIQPENLE	1.5482	Have similarities with cell surface proteins (plasma membrane protein)
LTMNNK	2.5695	Have similarities with cell surface proteins (glutamate receptor)
TGTPHWNNK	1.6563	Have similarities with cell surface proteins (transmembrane receptor)
AHAKRQ	1.1540	Has nothing in common with cell surface proteins of human
ESTENSKM	0.7702	Have similarities with cell surface proteins (kinase protein)

Based on the analysis steps that have been carried out, 3 epitopes with the higesth antigenicity score from 3 different epitope mapping method were obtained that can be used as peptide vaccine candidates.

Table 3. Epitope Candidate with Kolaskar and Tongaonkar Method, Bepipred, and Emini

Epitope Sequence	Amino Acid Position		Epitope Score	Epitope Mapping Method	Antigenicity analysis	Similarity Analysis
	Start	*End*				
SLGLDCE	27	33	1.074	Kolaskar & Tangaonkar	Probable Antigen (1.7129)	Has nothing in common with cell surface proteins of human
ETDENRAKVEV TPNSPRAEATLG	1	23	2.031	Bepipred	Probable Antigen (0.6092)	
AHAKRQ	90	95	2.084	Emini	Probable Antigen (1.1540)	

Note: start: the initial location of the sequence in the amino acid sequence, end: the location of the end of the sequence in the amino acid sequence

B cells and T cells do not recognize the pathogen as a whole but through the molecular component, namely the antigen. Specific receptors will recognize antigens on the surface of B and T cells. The recognition of antigens by B and T cells is very different. B-cells recognize solvent-exposed antigens through antigen receptors, referred to as B-cell receptors (BCR), consisting of membrane-bound immunoglobulins. Upon activation, B-cells differentiate and secrete soluble forms of immunoglobulins, known as antibodies, and play a role in mediating humoral adaptive immunity. Antibodies released by B-cells can have different functions which are triggered upon binding to their cognate antigens. These functions include neutralizing toxins and pathogens as well as labeling them for destruction. The B cell epitope is the part of the antigen that binds to the immunoglobulin or antibody. These epitopes recognized by B cells can be solvent regions exposed to the antigen and have different chemical properties. However, most antigens are proteins and are subject to epitope prediction (Sanchez-Trincado et al., 2017). After obtaining the candidate epitope, this protein can be developed into a vaccine through the particular delivery system such as lipid nanoparticle[23].

CONCLUSION

Based on the research results, ZIKV protein E can be used as a B cell epitope, namely SLGLDCE with a length of 7 amino acids, ETDENRAKVEVTPNSPRAEATLG with an amino acid length of 23, and AHAKRQ with an amino acid length of 6. This epitope candidate has a high antigenicity score and has no resemblance to human body surface receptors so that it is expected to be a vaccine candidate.

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