JURNAL ILMIAH BERKALA SAINS DAN TERAPAN KIMIA

Jurnal Ilmiah Berkala: Sains dan Terapan Kimia
Vol. 16 No. 1, Januari 2022

Design of Vaccines Candidate Based on Ebola Virus Epitop with In-Silico Approach

Perancangan Kandidat Vaksin Berbasis Epitop Virus Ebola dengan Pendekatan *In-Silico*

Erwin Prasetya Toepak[1], Sari Namarito Simarmata[1,*], Sudarman Rahman[1], Stevin Carolius Angga[1]

[1]Chemistry Study Program, Faculty of Mathematics and Natural Sciences, University of Palangka Raya
Kampus UPR Tunjung Nyaho, Palangka Raya, 73111, Indonesia

**Email: sarinamarito@gmail.com*

ABSTRACT

The World Health Organization (WHO) recorded as many as 2,299 cases of death with a case percentage of 66% during 2018 to 2020 in the Democratic Republic of the Congo due to the Ebola virus. Ebola virus is a member of the Filoviridae family. One of the viro cores consists of glycoprotein (GP). Messenger RNA (mRNA) generates long GP chains for the attachment protein (GP 1) and the fusion protein (GP 2). Epitope-based vaccines are a promising approach because epitopes represent immunogenic regions that elicit immunity specifically. Epitope prediction was performed based on the GP EBOV sequence available in the Data Bank. The designed vaccine could be one of the candidates for the Ebola virus vaccine. The design of the virus with access to NCBI AAB81004 was carried out by testing such as B cell epitope, T cell, and their antigenicity using the VaxiJen v2.0 server and IEDB. The T cell epitope prediction results showed that 20 T cell epitopes interacted with the Major Histocompatibility Complex (MHC) with the highest score of 2.8069. B cell epitope by linear BepiPred assay had 77 candidate epitope peptides from sequence 401-477. Karplus and Schulz's flexibility predictions showed a predictive value of 1.119 with a threshold of 1.008, with the analyzed area having an antigenic tendency where the threshold area was yellow.

Keywords: Ebolavirus; Vaccine; Epitope B Cell; Epitope T Cell; In Silico

ABSTRAK

World Health Organization (WHO) mencatat sebanyak 2.299 kasus meninggal dengan persentase kasus 66% pada tahun 2018-2020 di Republik Demokrasi Kongo akibat virus Ebola. Ebolavirus merupakan salah satu anggota family Filoviridae. Inti viron salah satunya terdiri dari glikoprotein (GP). RNA messenger (mRNA) menghasilkan rantai panjang GP untuk protein pelekat (GP 1) dan protein fusi (GP 2). Vaksin berasis epitop sebagai salah satu pendekatan yang menjanjikan dikarenakan epitop mewakili wilayah imunogenik yang dapat secara khusus memunculkan kekebalan. Prediksi epitop dilakukan berdasarkan urutan GP EBOV yang tersedia pada Bank Data. Vaksin yang dirancang dapat dijadikan salah satu kandidat vaksin virus Ebola. Perancangan virus dengan akses NCBI AAB81004 dilakukan dengan pengujian seperti epitop sel B, sel T, serta antegenitasnya menggunakan server VaxiJen v2.0 dan IEDB. Hasil prediksi epitop sel T memperlihatkan terdapat 20 epitop sel T yang berinteraksi dengan Major Hystocompatibility Complex (MHC) dengan skor tertinggi 2.8069, epitop sel B dengan pengujian BepiPred linier memiliki kandidat epitop banyak 77 pepttida dari urutan 401-477. Prediksi Karplus dan Schulz flexibility menunjukkan nilai prediksi 1,119 dengan ambang batas 1,008 dengan daerah yang dianalisis memiliki kecenderungan antigenik dimana daerah ambang batasnya berwarna kuning.

Kata Kunci: Ebolavirus; Vaksin; Epitop Sel B; Epitop Sel T; In Silico

Submitted: December 22, 2021; **Accepted**: February 22, 2022: **Available online**: March 8, 2022

P-ISSN: 1411-1616, E-ISSN: 2549-8215

1. INTRODUCTION

Based on data from the World Health Organization (WHO), there were 3,481 cases and 2,299 deaths with a case percentage of 66% in 2018-2020 in the Democratic Republic of Congo due to the Ebola virus and continues to this day. The Ebola virus is one of the largest outbreaks, with many deaths and a significant increase in cases compared to other outbreaks. The first Ebola virus disease was discovered in 1976 by Dr. Peter Piotin in Zire, Africa. In tropical Saharan Africa, additional Ebola virus disease (EVB) outbreaks have occurred between humans (Moghadam, Omidi, Bayrami, Moghadam, & SeyedAlinaghi, 2015; Rajak, Jain, Singh, Sharma, & Dixit, 2015; Zawilińska & Kosz-Vnenchak, 2014).

Ebola virus is a member of the order Mononegavirales, the Filoviridae family consisting of five species, including Zaire Ebolavirus, Bundibugyo Ebolavirus, Sudan Ebolavirus, Reston Ebolavirus, and Tai Forest Ebolavirus (Sridhar, 2015; Zawilińska & Kosz-Vnenchak, 2014). The negative RNA genome encodes 7 proteins. The viron core consists of an RNA-independent genome, RNA-polymerase (L), nucleoprotein (NP) binding to genomic RNA, and viral nucleocapsid proteins (VP35, VP40, VP30, and VP24) surrounded by a lipid envelope with surface projections consisting of glycoproteins. GPs have multimeric surfaces derived from single structural GPs that function in cell attachment, fusion, and cell entry (Dash et al., 2017; Sridhar, 2015; Zawilińska & Kosz-Vnenchak, 2014).

The GP gene performs different transcriptions of glycoproteins. RNA messenger (mRNA) generates long GP chains for the attachment protein (GP1) and the fusion protein (GP2) containing additional adenosine. RNA transcription results in synthesized soluble GP (sGP). In contrast, small soluble GP (ssGP) added 2 adenosine residues during the process (Dash et al., 2017). GP is virally described as a virion surface that has the role of catalyzing membrane fusion and amalgamation into host cells. It indicates this component's importance for vaccines and as a potential target for developing attachment inhibitors and antibodies (Dash et al., 2017).

The development of the Ebola vaccine is carried out by inactivating the virus. Several methods can be used to manufacture vaccines, such as DNA vaccine, viral recombination, protein subunits, protein recombination, and VLP. All vaccines manufactured must undergo clinical trials to evaluate their reactogenicity and immunogenicity. Several vaccines in clinical trials target the GP of the Ebola virus and provide a dominant immune response (Sridhar, 2015).

Vaccines are antigenic given to stimulate the immune system to prevent viral infections. Immune system stimulation involves T cell epitopes and B cell epitopes. Both of these epitopes have responsibilities in cellular and humoral immunity (Patronov & Doytchinova, 2013; Sanchez-Trincado, Gomez-Perosanz, & Reche, 2017).

Epitope vaccines refer to alternative vaccine strategies that use peptide fragments to elicit a strong immune response against a pathogen instead of using the whole organism. The application of epitope vaccines is growing with advances in biotechnology due to in silico tools and databases that provide safe and promising results (Garg, Srivastava, & Srivastava, 2020). Despite the tremendous progress in the last few decades, there is no generally accepted strategy for implementing a vaccine design approach. From different methods, epitope-based vaccines have been developed to provide promising results for prophylactic and therapeutic aspects of pathogen-specific immunity. This strategy offers several advantages, including eliminating unwanted immune responses by designing specific constructs, establishing prolonged immunity with the required response, and cost and time

effectiveness (Parvizpour, Pourseif, Razmara, Rafi, & Omidi, 2020).

Epitope-based vaccines are a promising approach. Epitopes represent protein sequences' immunogenic regions that elicit accurate immunity (Dash et al., 2017). Epitope prediction was carried out based on the GP Ebov sequence available in the in silico-based National Center for Biotechnology Information (NCBI) Data Bank. Epitope vaccine designed based on this glycoprotein could be used as a candidate for the Ebola virus vaccine through several testing parameters such as prediction of B cell epitope, T cell epitope, and antigenicity.

2. MATERIALS AND METHODS

2.1. Materials

The GP EBOV protein sequences were taken from the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/) glycoprotein [Zaire ebolavirus], GenBank: AAB81004.1. The protein sequences were created in FASTA format. Epitope prediction tests for B cells, T cells, and antigenicity were carried out using the VaxiJen v2.0 server (https://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) and IEDB (https://www.iedb.org/). B cell epitope was tested with BepiPred 2.0 and Karpluz and Schulz Flexibility, while T cell epitope was tested with NetCTL v1.2 server (https://services.healthtech.dtu.dk/service.php?NetCTL-1.2).

2.2. Methods

In the NCBI, the protein was selected, and the Zaire Ebolavirus glycoprotein was entered, then the GenBank access number: AAB81004.1 was selected. The glycoprotein [Zaire ebolavirus] sequence, GenBank: AAB81004.1, was prepared in FASTA format then tested using VaxiJen v2.0 to determine antigenicity with a threshold of 0.5. The protein sequences were then tested using the IEDB. On the IEDB web server, select B Cell Epitope Prediction, protein sequences in FASTA format were entered, and then predictions were selected using BepiPred. The same steps were carried out for testing with Karplus and Schulz Flexibility. T cell epitopes were assayed with NetCTL to predict binding between T cells and MHC.

3. RESULTS AND DISCUSSION

The Zaire Ebolavirus GP sequence is shown in Fig. 1. The antigenicity test was performed using the VaxiJen v2.0 server with the FASTA format protein sequence. The test was carried out with a threshold value of 0.5. Determination of this value aims to see the accuracy and sensitivity of the test. Fig. 2 showes the predictive value of 0.4946 antigenicity. Prediction of MHC-bound T cell epitope was performed with NetCTL. The test results presented in Table 1. B cell epitope prediction was performed using IEDB with linear BepiPred prediction to determine B cell epitope (Table 2) and Karpluz and Schulz Flexibility (Fig. 3). This prediction was chosen because it displays the results of the flexibility of the B cell epitope region. The maximum predicted value was 1.119 with a threshold of 1.008.

The general idea behind peptide vaccines is based on a chemical approach for synthesizing B cell and T cell epitopes which were identified as being immunodominant and able to induce a specific immune response. The B cell epitope of the target molecule can be conjugated with the T cell epitope to form immunogenic. T cell epitopes bind to MHC to induce an immune response. T cells can trigger specific adaptive immunity for pathogens. Epitope recognition by T cells and induction of immune response have key roles for the individual immune system.

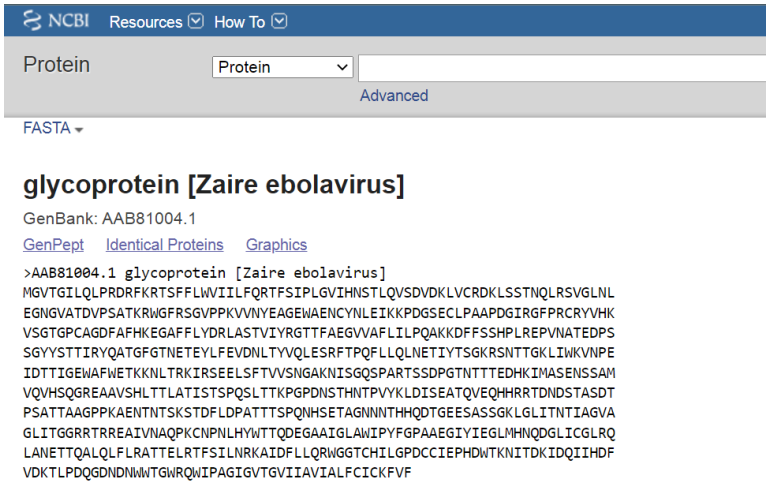
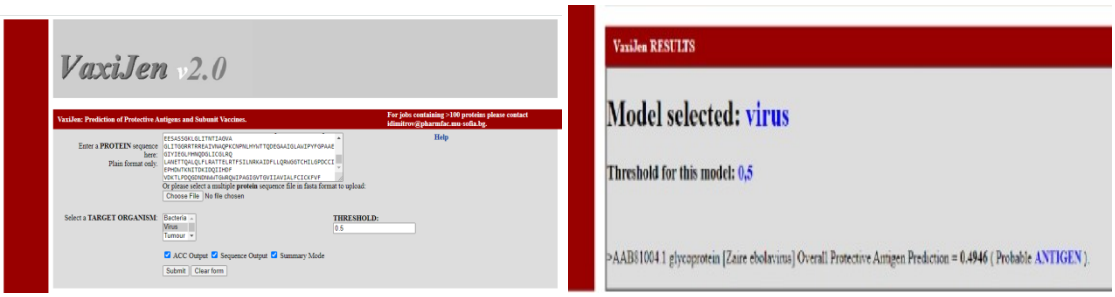


Figure 1. [Zaire ebolavirus] glycoprotein sequence, GenBank: AAB81004.1



(a) (b)

Figure 2. Protein sequence input (a), and GP sequence antigenic test results with a threshold of 0.5 using VaxiJen v2.0 (b)

Table 1. Prediction results of T cell epitope interacting with MHC using NetCTL

Peptide	Skor	Peptide	Skor
ATEDPSSGY	2.8069	STSPQSLTT	0.8530
NSTHNTPVY	1.6532	NTSKSTDFL	0.8769
TEDPSSGYY	1.5503	RTSFFLWI	0.8393
ITDKIDQII	1.0729	LLQLNETIY	0.9753
SSDPGTNTT	0.9700	TTGKLIWKV	0.8119
LFEVDNLTY	1.1224	RSEELSFTV	0.8030
TTQALQLFL	0.9347	ATDVPSATK	0.8179
RTDNDSTAS	0.7621	KIDQIIHDF	0.8565
ASENSSAMV	0.9823	GTNETELYF	0.8084
HKEGAFFLY	1.0887	AIGLAWIPY	0.8453

T cell epitope prediction aims to identify the shortest peptide in the antigen capable of inducing CD4 or CD8 T cells. MHC-peptide binding is the most selective in determining T cell epitopes. Data-driven methods for predicting MHC-peptide binding are based on peptide sequences known to bind to MHC molecules. Antigen processing forms a repertoire of peptides available for T cell binding (Sanchez-Trincado et al., 2017). In Table 1, data on glycoprotein peptides bound to MHC are presented. The results showed that 20 peptides could bind to MHC. Among the T cell epitopes produced by the ATEDPSSGY peptide with a value of 2.8069 is a candidate peptide for the Ebola vaccine because it has the highest value.

Table 2. Prediction of B cell epitope using BepiPred Linear

No	Initiall	End	Peptide	Wavelength
1	14	14	F	1
2	57	59	LSS	3
3	73	106	NGVATDVPSATRWGFRSGVPPKVVNYEAGEWAE	34
4	114	131	KKPDGSECLPAAPDGIRG	18
5	141	148	VSGTGPCA	8
6	175	176	TF	2
7	191	193	KDF	3
8	198	215	PLREPVNATEDPSSGYYS	18
9	223	229	TGFGTNE	7
10	261	270	YTSGKRSNTT	10
11	279	285	PEIDTTI	7
12	296	296	N	1
13	313	339	NGAKNISGQSPARTSSDPGTNTTTEDH	27
14	341	342	IM	2
15	344	348	SENSS	5
16	355	360	SQGREA	6
17	371	393	ISTSPQSLTTKPGPDNSTHNTPV	23
18	401	477	ATQVEQHHRRTDNDSTASDTPSATTAAGPPKAENTNTSKSTDFLDPATTTSP QNHSETAGNNNTHHQDTGEESASSG	77
19	497	500	RRTR	4
20	506	513	NAQPKCNP	8
21	518	526	WTTQDEGAA	9
22	536	540	GPAAE	5
23	563	564	NE	2
24	566	566	T	1
25	609	611	CIE	3
26	613	618	HDWTKN	6
27	620	621	TD	2
28	633	646	KTLPDQGDNDNWWT	14

B cell epitope prediction aims to facilitate the identification of B cell epitope for antibody production. Linear B cell epitopes consist of sequential peptide residues. Antibodies that recognize linear B cell epitopes can recognize antigens. Although B cell epitopes are in the minority, this prediction has received significant attention because B cell epitopes have a role in binding antibodies produced. Epitope prediction by computation is based on a simple amino acid propensity scale that describes the physicochemical features of B cells (Sanchez-Tricando et al., 2017).

In the B cell epitope test with the prediction of BepiPred linear B cells with a threshold of 0.35, the maximum value was 2.514. The peptide residues constituting the B cell epitope are presented in Table 2. Tests with Karpluz and Schulz predictions showed the flexibility of the B cell epitope presented in Fig. 3 with a maximum value of 1119 and a more dominant yellow region. The yellow color indicates an antigenic tendency in the region above the threshold. B cell epitopes induce minimal immunity that is strong enough to provide a humoral immune response and does not cause harmful side effects to the body. Epitope-based vaccine manufacture also has the advantage of eliciting an accurate immune response because the epitope is considered an immunogenic region (Dash et al., 2017). The flexibility of the peptide is correlated with the antigenicity. Therefore, Karpluz and Schulz were used to show the flexibility of the peptide. In Fig. 3, the Y-axis represents the score, and the X-axis represents the length of the sequence.

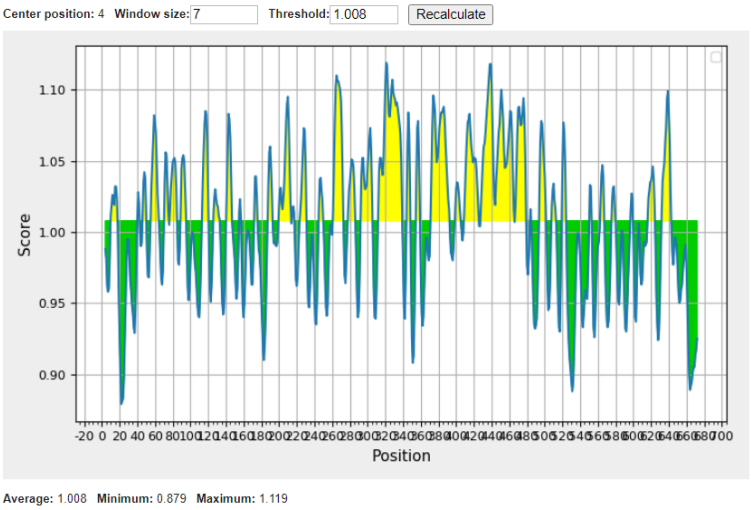


Figure 3. Prediction results of B cell epitope using IEDB with Karpluz and Schulz Flexibility prediction. The yellow color indicates the antigenic area, while the green indicates the peptide that does not cross the threshold.

4. CONCLUSIONS

Antigenicity test showed a prediction of antigen on glycoprotein with a value of 0.4946. There were 20 T cell epitopes with a score of 2.8069 with the peptide sequence ATEDPSSGY. B cell epitope with Karpluz and Schulz Flexibility test showed a predictive result of 1,119. In the BepiPred test, there were 77 candidate B cell epitopes of the order 401-477 (ATQVEQHHRRTDNDSTASDTPSATTA AGPPKAENTNTSKSTDFLDOATTTSPQN HSETAGNNNTHHQDTGEESASSG). Flexibility test results have an antigenic tendency that is above the yellow threshold. Between the epitope of B cells and T cells produced, there is the same peptide, namely SSG, making it a candidate for vaccine design for the Ebola virus.

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