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Original Research Article

In Silico Analysis Prediction of B-Cell Epitope as a Vaccine Candidate for SARS-CoV-2 B.1.617.2 (Delta) Variant

Mauritz Nicolaas Wattimena*, Wijanarka Wijanarka

Department of Biology, Faculty of Science and Mathematics, Universitas Diponegoro, Semarang, Indonesia

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Abstract

Background: The COVID-19 pandemic by SARS-CoV-2 has caused many losses. One way to prevent the spread of this virus is to get vaccinated. However, the latest SARS-CoV-2 variants, including variant B.1.617.2 (Delta) are doubtful to be inhibited by existing vaccines because of mutations. Therefore, we need a new vaccine candidate that is effective against this SARS-CoV-2 variant. Through an immunoinformatics approach with various software and analysis websites, vaccine candidates can be predicted in a short time.

Objective: Identity, analyze, obtain, and confirm the selected B-cell epitope sequence that can be used as a vaccine candidate for the SARS-CoV-2 B.1.617.2 (Delta) variant.

Methods: This research was conducted by isolating the amino acid peptide sequence in the SARS-CoV-2 B.1.617.2 (Delta) variant protein spike from the Protein Data Bank which is suspected to be an immunogenic epitope and can be used as a vaccine candidate. A Series of tests were carried out such as antigenicity, toxicity, allergenicity, and BLAST® protein to ensure that this vaccine candidate is safe for later application into the human body. The next stage is a conservation analysis to see its potential by comparing it with the SARS-CoV-2 Delta (B.1.617.2) variant spike protein sequence in Indonesia. The study ended by mapping amino acid peptides to the SARS-CoV-2 Delta (B.1.617.2) variant spike protein using the Biovia Discovery Studio Visualizer v21.1.0.20298 2020 software to ensure that the selected sequences were epitope.

Results: From the five amino acid peptides that have been isolated, the FTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFT epitope sequence has good results than the others. It is probable an antigen, non-toxic, non-allergen, and non-homolog to the human body protein. Moreover, this peptide knew eligible to use as the vaccine candidate, either in Indonesia or overseas. This peptide also still promising enough to work against the SARS-CoV-2 B.1.1.529 (Omicron) variant.

Conclusion: Based on this in silico study, it was found that the FTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFT epitope sequence was the best to be used as a vaccine candidate of SARS-CoV-2 B.1.617.2 (Delta) variant.

Keywords: *SARS-CoV-2 B.1.617.2 (Delta) variant; B-cell epitope; vaccine; in silico immunoinformatics.*
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INTRODUCTION

The Corona Virus Disease-19 (COVID-19) pandemic has swept the world for the past one and a half years. This pandemic is caused by the Severe Acute Respiratory Syndrome Corona Virus-2 (SARS-CoV-2) virus which attacks the respiratory system acutely in sufferers.

* Corresponding author:
E-mail: *mauritznicolaas15@gmail.com*
(Mauritz Nicolaas Wattimena)

People infected with SARS-CoV-2 will show signs such as fever, cough, dyspnea, muscle and chest pain, headache and throat, diarrhea, and other serious complications.[1,2]

The state of the COVID-19 pandemic is likely to be exacerbated by the behavior patterns of people who are careless and do not maintain health protocols as recommended by the government. Therefore, there has been a spike in COVID-19 cases in the world in general and Indonesia in particular in 2021. This was then exacerbated by the mutation of the SARS-CoV-2 virus that caused the COVID-19 pandemic, which led to the emergence of various new variants in nature. These new variants of SARS-CoV-2 in nature are known to be more deadly than the previous types that already exist. These variants include B.1.1.7 (Alfa), B.1.351 (Beta), P.1 (Gamma), and various other types of SARS-CoV-2 variants including B.1.617.2 or commonly known as Delta.[3,4]

Variant B.1.617.2 (Delta) SARS-Cov-2 is a variant that first appeared in India in April 2021. In this variant, 12 point mutations were found in the spike protein (S protein) when compared to the wild type SARS-CoV-2 which was first detected in Wuhan, China last December 2019.[5] This variant is known to have a higher level of spread when compared to variants B.1.1.7 or Alfa. Data from the UK public health found that this Delta variant was rapidly spreading, causing around 5,472 new cases in the week to 26 May 2021, and even being the dominant strain when compared to the alpha variant which was first discovered in the country itself. This variant spreads rapidly through schools among children aged 7-11 years and people who refuse vaccination.[6] Variant B.1.617.2 (Delta) which is known to have a fairly high level of spread raises the question of whether the vaccination activities carried out can minimize the spread of this variant or not. According to research conducted by Bernal et al. (2021), it was found that the effectiveness of the ChAdOx1 nCoV-19 vaccine to prevent the SARS-CoV-2 infection after the first dose was lower among people infected with the delta variant (30.7%) when compared to those infected with the alpha variant (48.7%). In people who are infected with the delta variant but have received 2 doses of the ChAdOx1 nCoV-2 vaccine, the effectiveness value is only around 67.0%.[7] Research conducted by Fowlkes et al. (2021) proved that from a total of 2,875 participants in the United States who had been fully vaccinated before the presence of the Delta variant (with the Pfizer-BioNTech, Moderna, Janssen Johnson & Johnson vaccine) the vaccine effectiveness value to prevent the SARS-CoV-2 infection was in the range of 91%. However, after the Delta variant was present, the effectiveness of the vaccine decreased to 66%.[8] Research conducted by Chen et al. (2021) showed the predicted efficacy of CoronaVac against the Delta variant that caused severe disease only at 75.3%.[9] This needs to be studied further whether the currently available vaccine is still effective enough to prevent SARS-CoV-2 variant B.1.617.2 (Delta) or not.

Therefore, based on the above facts, the researcher intends to seek, identify, and assess a vaccine candidate that is immunogenic and capable of becoming an inhibitor of SARS-CoV-2, especially for variant B.1.617.2 (Delta). This vaccine candidate uses B cell epitope through an in silico immunoinformatics approach. The immunoinformatics approach that emphasizes the design of B cell epitope potential mapping algorithm studies is carried out because it has various advantages, including because it requires more efficient time, reduces vaccine development costs, and many related studies have been carried out and succeeded. The use of B cell epitopes in this study is also due to the function of B cells themselves which are known to be able to recognize specific antigens and produce antibodies. According to Lervani (2018), as part of the adaptive immune response, B cells play an important role in maintaining the body's defense against pathogens. B cells can produce antibodies that are important as a major element of the human immune response and can recognize specific antigens.[10] So far, studies of epitopes, both B and T cells, have been used for various needs for vaccine products[11] such as dengue fever and measles.[12] However, until now, it is known that there has been no further and specific research in utilizing this B cell epitope as a candidate for the SARS-CoV-2 vaccine variant B.1.617.2 (Delta). The closest research that has been carried out in-depth is that of Yang et al. (2021) compared potential B cell epitope sequences between SARS-CoV-2 wild type strain, D614G variant, alpha and beta, and SARS-CoV.[13]

Based on the above background, the researcher hereby intends to conduct a study using an immunoinformatics approach regarding the *In Silico Analysis Prediction of B-Cell Epitope as a Vaccine Candidate for SARS-CoV-2 B.1.617.2 (Delta) Variant*. This study aimed to obtain the epitope sequence of the SARS-CoV-2 variant Delta (B.1.617.2) spike protein which can be used as a vaccine candidate. The expected benefit from this research is the acquisition of B cell epitope sequences that can ward off the SARS-CoV-2 variant which is likely to still have the potential to endanger human life in the future.

MATERIALS AND METHODS

This research was going on September-November 2021 online-virtually from the Research Center of Molecular Biotechnology and Bioinformatics Padjadjaran University, Jatinangor, West Java. This study is an experimental research and uses the 3D structure of SARS-CoV-2 B.1.617.2 (Delta) protein spike as the primary data taken from *Protein Data Bank (PDB)* (https://www.rcsb.org/). The data sequence of SARS-CoV-2 B.1.617.2 (Delta) spike protein in Indonesia for comparison in *Epitope Conservancy Analysis* was collected from *the GISAID Epi-CoV™ Database* website (https://www.epicov.org/epi3/frontend), then cleaned in *MEGA software application*, so can be used for the next method.

Materials that used in this study are Notebook Personal Computer (PC) ASUS® Laptop X441MA with the specification processor Intel(R) Celeron(R) N4000 CPU @ 1.10GHz, RAM 4.00 GB, internal storage 1 TB, and Windows 11 as the operating system, software application *MEGA Version 10.1.8 2021*, and *Biovia Discovery Studio Visualizer v21.1.0.20298 2020*. Data analysis methods were conducted based on the method

from Kolaskar & Tongaonkar (1990) with modifications as follows[14]:

1. Determination of the best linear epitope collected from the SARS-CoV-2 variant B.1.617.2 spike protein sequence was carried out through the *Ellipro website: Antibody Epitope Prediction* (http://tools.iedb.org/ellipro/) by entering the PDB ID of the spike protein. SARS-CoV-2 variant B.1.617.2 (Delta) was previously obtained from Protein Data Bank.
2. The antigenicity test was carried out using the *Vaxi Jen v.2* websites (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html). The selected peptide sequences are then copied one by one and entered into the available search field. After that, "virus" is selected as the target organism, and then the "submit" button is pressed. Once loaded, the results of the antigenicity prediction on the page will be raised.
3. The toxicity test was carried out with the help of the *ToxinPred* website (https://webs.iiitd.edu.in/raghava/toxinpred/design.php). The pre-selected epitope suspected amino acid peptide sequences were entered into the search field. Then, by pressing the "Run Analysis" button, you will get the toxicity prediction results from selected amino acid peptide sequences.
4. Allergenicity test was carried out using the *AllerTOP v.* 2.0 website (https://www.ddg-pharmfac.net/AllerTOP/index.html). The selected epitope sequence is then entered into the available search field. Then by pressing the "Get the result" button, you will find the allergenicity prediction results from the sequence selected epitope.
5. The BLAST® protein assay was performed using the *NCBI BLAST*® website (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome). The selected peptide sequence is entered in the search field, then in the "organism" section, it is listed "Homo sapiens" to see its identity with proteins in the human body. After that, the BLAST button is clicked and the page will contain a list of identified proteins between the peptides that we have entered and those in the human body.
6. The conservation analysis was using the *Epitope Conservancy Analysis* website (http://tools.iedb.org/conservancy/). Conservation analysis was carried out by entering all previously selected peptide sequences of suspected epitope and spike protein sequences that had also been obtained

Table 1. Epitope linear prediction via ElliPro: Antibody Epitope Prediction website

Start	End	Peptides	Number of residues	Score
14	29	QCVNLRTRTQLPPAYT	16	0.811
236	264	FQTLLALHRSAAAYY	15	0.794
92	106	FASTEKSNIIRGWIF	15	0.743
328	361	PNITNLCPFGEVFNATRFASVYAWNRKRISNCVA	34	0.726
390	428	FTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFT	39	0.706

Table 2. Antigenicity test by *VaxiJen v.20* website

Peptide Sequence	Antigenicity Prediction Score	Probability
QCVNLRTRTQLPPAYT	1,2827	Antigen
FQTLLALHRSAAAYY	0.2091	Non-Antigen
FASTEKSNIIRGWIF	-0.4611	Non-Antigen
PNITNLCPFGEVFNATRFASVYAWNRKRISNCVA	0.4611	Antigen
FTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFT	0.5922	Antigen

Note: threshold score: 0.4

Table 3. Toxicity and hydrophilicity test of selected peptide sequences suspected epitope via *ToxinPred* website

Peptide Sequence	Mutation Position	Prediction	Hydrophilicity
QCVNLRTRTQLPPAYT	No mutation	No Toxin	-0.22
FQTLLALHRSAAAYY	No mutation	No Toxin	-0.79
FASTEKSNIIRGWIF	No mutation	No Toxin	-0.33
PNITNLCPFGEVFNATRFASVYAWNRKRISNCVA	No mutation	No Toxin	-0.33
FTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFT	No mutation	No Toxin	0.06

Table 4. Allergenicity test through *AllerTOP v. 2.0* website

Peptide Sequence	Probability
QCVNLRTRTQLPPAYT	Non-allergen
FQTLLALHRSAAAYY	Allergen
FASTEKSNIIRGWIF	Non-allergen
PNITNLCPFGEVFNATRFASVYAWNRKRISNCVA	Allergen
FTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFT	Non-allergen

in the provided column. The next step is then the "submit" button is pressed and after that, the page will load the results of the conservation analysis of the suspected epitope peptide sequence against the SARS-CoV-2 B.1.671.2 (Delta) variant in Indonesia.

7. The final step taken to ensure that the selected peptide is an epitope is to map it using the Biovia Discovery Studio Visualizer v21.1.0.20298 software. The 3D structure of the "*Cryo-EM structure of SARS-CoV-2 S-Delta variant (B.1.617) .2), two RBD-up conformation 1*" previously collected from PDB are then displayed in this software. After that, the appearance of the protein was adjusted for color and structure to facilitate the observation process. The next step is to map the selected peptides by revealing the aromatic surface elements. These peptides can be ascertained to be epitopes in the SARS-CoV-2 spike protein variant B.1.617.2 (Delta) if there is an appropriate aromatic residue site with the characteristics of an epitope.

RESULTS

Based on the prediction of linear epitope isolated from the SARS-CoV-2 B.1.617.2 variant spike protein sequence which was carried out through the Ellipro website *Antibody Epitope Prediction* (http://tools.iedb.org/ellipro/), 5 amino acid peptides were taken. best with residue count <50 due to the limitations of the *ToxinPred* software for toxicity testing (https://webs.iiitd.edu.in/raghava/toxinpred/design.php) which can only count sequences under 50 residues. The prediction results are then accumulated into one data as listed in Table 1.

The antigenicity test carried out through the *VaxiJen v.2* website (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) showed mixed results on the five amino acid peptide sequences suspected to be epitope as listed in Table 2.

Toxicity and hydrophilicity tests were carried out with the help of the *ToxinPred* website (https://webs.iiitd.edu.in/raghava/toxinpred/design.php). Results peptide assays are listed as in Table 3.

Based on the allergy test that has been carried out using the *AllerTOP v 2.0. Bioinformatics tool for allergenicity prediction* website (https://www.ddg-pharmfac.net/AllerTOP/index.html), various results were obtained among the five selected epitope candidates such as listed in Table 4.

The BLAST® protein assay was conducted through the *NCBI BLAST*® website (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&PAGETYPE=BlastSearch&LINKLOC=blasthome) showed 100% dominant results as listed in Table 5.

Conservation analysis conducted using the *Epitope Conservancy Analysis* website (http://tools.iedb.org/conservancy/) was found to be different in the five selected epitopes as listed in Table 6.

Based on the results shown in Table 7 below, we know that there is no real difference between the sequence

Table 5. Homologous protein assay via *BLAST® NCBI* website

Peptide Sequence	% Identity
QCVNLRTRTQLPPAYT	100%
FQTLLALHRSAAAYY	100%
FASTEKSNIIRGWIF	100%
PNITNLCPFGEVFNATRFASVYAWNRKRISNCVA	100%
FTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFT	68,75%

Table 6. Conservation analysis of selected peptides in Indonesia through the *Epitope Conservancy Analysis* website

Peptide Sequence	% Protein Sequence Identification (≤100)
QCVNLRTRTQLPPAYT	33,71%
FQTLLALHRSAAAYY	0,00%
FASTEKSNIIRGWIF	98,88%
PNITNLCPFGEVFNATRFASVYAWNRKRISNCVA	99,63%
FTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFT	99,63%

Table 7. Comparison between the selected peptides and the random samples of SARS-CoV-2 B.1.617.2 (Delta) variant around the world

Selected peptides	India	Indonesia	United states	Brazil	France	Kenya
QCVNLRTRTQLPPAYT	*	*	*	*	*	*
FQTLLALHRSAAAYY	..HRSYLTPG	..HRSYLTPG	..HRSYLTPG	..HRSYLTPG	..HRSYLTPG	..HRSYLTPG
FASTEKSNIIRGWIF	*	*	*	*	*	*
PNITNLCPFGEVFNATRFASVYAWNRKRISNCVA	*	*	*	*	*	*
FTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFT	*	*	*	*	*	*

between the Indonesia sequence and another sample around the world, especially in the selected peptides region. From the table below, we also know that these selected peptides are eligible for being a vaccine candidate, either for Indonesia itself or to another country. Spike protein sequence random samples around the world were collected from the GISAID Epi-CoV™ Database website (https://www.epicov.org/epi3/frontend). Spike protein sequence that has been collected, then aligned by the MEGA software application, compare with the Indonesia's that have been collected before, and then input into conservancy analysis website (http://tools.iedb.org/conservancy/).

DISCUSSION

Cell-B Epitope Prediction

The spike protein sequence of SARS-CoV-2 variant B.1.617.2 (Delta) was collected from the Protein Data Bank website. In the search field, search for the keyword "Spike Protein SARS-CoV-2 Delta Variant". After loading, select "*Cryo-EM structure of SARS-CoV-2 S-Delta variant (B.1.617.2), two RBD-up conformation 1*" (PDB ID: 7V7T). The reason for choosing the structure of SARS-CoV-2 with 2 RBD conforming upwards is based on the statement of McCallum et al. (2020) which states that because of that position, this antigen can be accessed by the ACE-2 receptor in the human body.[15] In addition, this is intended as an anticipatory demonstration step if there is a potential new antibody that can only target sites with conformational 2 RBD and above.

The five selected amino acid peptides are at values above 0.7 which indicated that the sequences are most likely a true epitope. According to Vita et al. (2015), the Immune Epitope Database (IEDB) website hosts epitope-specific experimental assays which mean each assay reflects antibody to the antigen or epitope being tested experimentally. Structures entered as restricted epitopes are those that were tested in the test or were deduced as epitopes by multiple sources. In most cases, these amino acid residue sequences are more epitope-containing regions. This means that the possible epitope can also not be limited only to a predetermined part. Epitope structures can be peptidic and non-peptidic. A peptidic epitope structure consists of linear and discontinuous amino acid sequences based on their position in the source protein. The peptidic epitope having 3D structural data is described in the presence of the residues found to be related to antibodies.[16]

Antigenicity and Allergenicity Test

Based on the tests that have been carried out, 3 out of 5 peptide sequences are predicted to have high antigenicity values. The high value of antigenicity in a peptide sequence is a marker of ideal properties possessed by vaccines. The higher the antigenicity value, the better its ability to stimulate B cells to form specific antibodies. Allergenicity test is a step carried out to test the selected vaccine candidate whether the peptide sequence to be used can cause allergies to the body or not. The ability not to create allergies for the body is also one of the ideal properties for a vaccine product. According to Rezaldi et al. (2021), a high value of antigenicity is one of the ideal characteristics that all vaccines should have. Allergy is the ability possessed by material to cause allergies. Non-allergen is an ideal trait that anti-viral vaccines should have. Antigenicity is the ability of an antigen to stimulate the formation of specific antibodies by B cells in the body.[17]

Toxicity Test

Based on the tests that have been carried out, the five peptide sequences are predicted to be non-toxic to the human body. Toxicity testing at the vaccine prediction stage is important to obtain a peptide that is not toxic when administered into the human body. According to Syakuran (2020), only peptides with negative toxicity values can be selected for further use. In addition, this test also found the value of hydrophilicity.[18] The higher the hydrophilicity value, the more certain the antigenic potential will be. This is following the statement of Sanchez-Trincado et al. (2017) which states that by calculating the hydrophilicity of residues for the prediction of B cell epitope, it can be seen their antigenic potential.[19] This is based on the assumption that the hydrophilic part is mainly located on the surface of the protein which will be directly accessible to the target antibody site.

BLAST® Protein Test

In BLAST® protein testing, the percent identical to protein in the human body of the 5th peptide sequence FTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKL PDDFT was found that the was smaller when compared to the others, which was only 68.75%. The smaller the percentage of identity, the better. According to Taupiqurrohman et al. (2016), epitope-based vaccines should not be homologous to proteins in the human body. This aims to avoid the occurrence of autoimmune. Autoimmunity is a condition where the specific immune response in the body can attack its cells. Epitope vaccines derived from similar (homologous) proteins such as those in humans are thought to cause this response. Autoimmune responses can usually be induced by T and B cells, respectively, or by both. In some cases, autoimmunity can result in loss of function of body tissues.[20]

According to Moody et al. (2021), due to its potential to cause autoantibodies responses including those associated with autoimmune diseases, the predicted epitopes were then compared to human proteins using the NCBI blast tool. Comprehensive mapping was done to discover its potential for cross-reactivity with self-antigens across multiple SARS-CoV-2. The NCBI blastp tool allowed us to apply criteria that not only relied on identical sequences but considered how similar charge and structure between amino acid variations may not impact antibody binding and therefore explore sequence similarity between the full length of the predicted epitopes and human proteins. So by this blasting phase, we could prevent the probability of the selected peptides causing autoimmune diseases in the human body ahead, before this vaccine candidate enters the in vitro phase (animal and pre-clinic). Computational methods, such as epitope mapping and blasting are useful techniques that can allow us to narrow questions and potential proteins

of interest in hypotheses before doing experimental studies.[21]

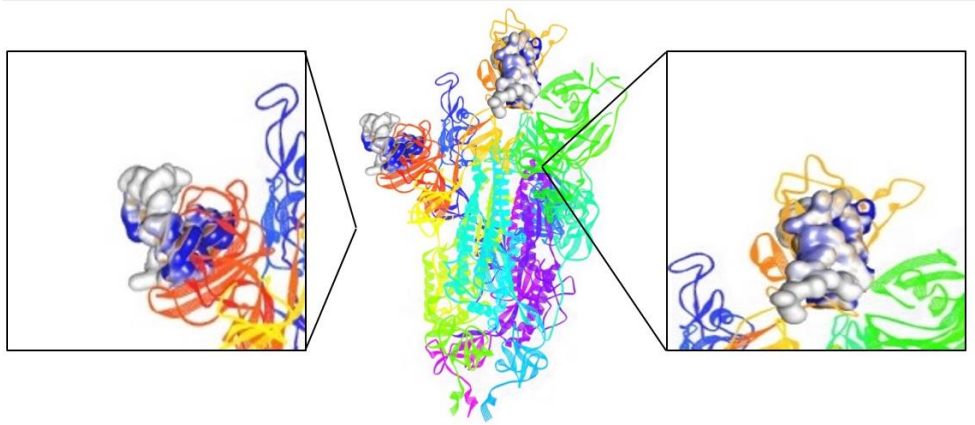

Figure 1. Mapping results for all selected peptides on the "*3D Cryo-EM structure of SARS-CoV-2 S-Delta variant (B.1.617.2), two RBD-up conformation 1*" (PDB ID: 7V7T) using Biovia Discovery Studio Visualizer v21.1.0.20298 software 2020

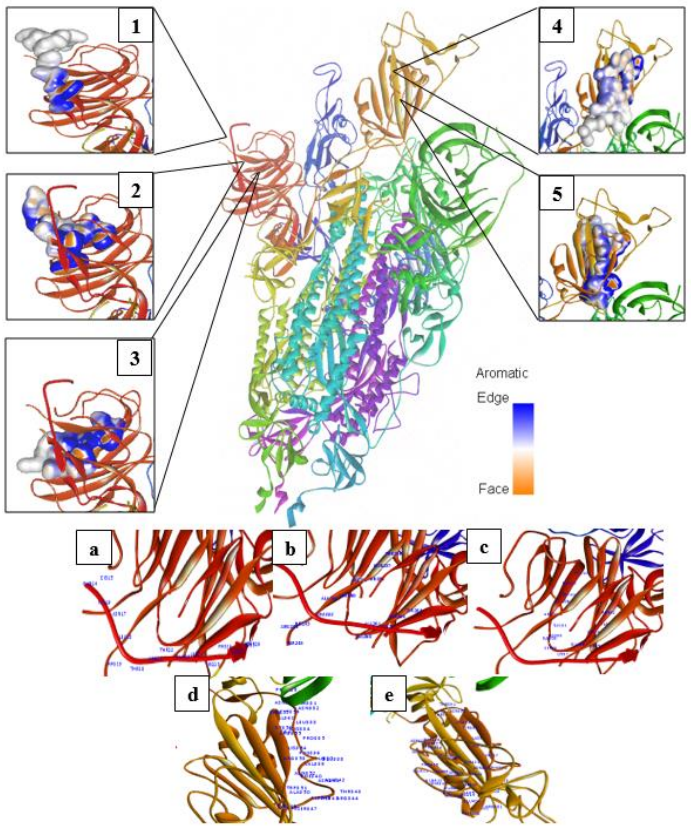

Figure 2. Selected peptide mapping results (1,a) QCVNLRTQLPPAY T; (2,b) FQTLLALHRSAAAYY; (3,c) FASTTEKSNIIRGWIF; (4,d) PNITNLCPFGEVFNATRFASVYAWNRKRISNCVA; and (5,e) FTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFT on "*3D Cryo-EM structure of SARS-CoV-2 S-Delta variant (B.1.617.2), two RBD-up conformation 1*" (PDB ID: 7V7T) using Biovia Discovery Studio software Visualizer v21.1.0.20298 2020

Conservation Analysis

The conservation analysis carried out showed quite good results, especially in 3 of the 5 selected protein-peptide sequences. The percentage identification of the selected protein sequence which is almost close to the value of 100% indicates that this peptide is quite conserved with the SARS-CoV-2 variant B.1.617.2 (Delta) which spreads in Indonesia. As many as 266 of the 267 total samples of the selected Delta variant virus in Indonesia are quite identical to the 2 amino acid protein peptides used, reaching a value of 99.63% so that they are quite suitable to be used as vaccine candidates in this country. According to Bui et al. (2007) conservation is defined as the fraction of a protein sequence containing the considered epitope at or above a certain (≥) identity level. The more the fraction of the protein sequence containing the epitope is below (<) a certain level of identity, the more it reflects the level of variability or uniqueness of the epitope.[22]

Mapping of Selected Peptides to the 3D Structure of Spike Protein SARS-CoV-2 Variant B.1.617.2 (Delta)

The mapping of selected amino acid peptides suspected to be epitopes to the 3D structure of the SARS-CoV-2 spike protein Variant B.1.617.2 (Delta) through the Biovia Discovery Studio Visualizer v21.1.0.20298 2020 software showed good results on the five selected amino acid residues. In Figure 1, it can be seen that the selected amino acid residues show an aromatic surface (which is bluish-grey). The characteristic of having an aromatic residue footprint is one of the characteristics that can indicate that the selected amino acid peptide is indeed an epitome of the SARS-CoV-2 spike protein variant B.1.617.2 (Delta). On the surface, residues are classified as epitopes if they are enriched with polar and aromatic residues and show a specific preference for the set of residue pairs.

Furthermore, if we look back at Figure 1, all the selected amino acid peptides are located in the S1 subunit and most of them are in the N-terminal and Receptor Binding Domain (RBD) region. Referring again to Table 4.1 above and by looking at the epitope mapping on the 3D structure of the Spike SARS-CoV-2 variant B.1.617.2 (Delta) protein in Figure 2 below, it can be seen that the amino acid peptide QCVNLRTQLPPAYT (sequences no. 14-29), FQTLLALHRSAAAYY (sequence no. 236-264), and FASTEKSNIIRGWIF (sequences no. 92-106) were in the N-terminal portion (residual sequences no. 14–305). Meanwhile, two other selected peptides, namely PNITNLCPFGEVFNATRFASVYAWNRKR ISNCVA (in the order of 328-361) and FTNVYADSFVIRGDEVRQIAPGQTGK IADYNYKLPDDFT (in the order of 390-428) are part of the Receptor Binding Domain (RBD) which is indeed in the order of 319–541.[23-25]

Based on the overall test results, the results obtained were quite diverse from the five amino acid peptides that had been selected and isolated from the Spike SARS-CoV-2 protein variant B.1.617.2 (Delta) as a vaccine candidate. The B cell epitope selected as a vaccine candidate for SARS-CoV-2 variant B.1.617.2 (Delta) is FTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKL PDDFT. This epitope is known to be in the sequence no. 390-428 so it is part of the Receptor Binding Domain (RBD). The RBD is commonly known as the human antibodies target because of its immunogenic region ability which binding to ACE2 receptors on the host cells.[26] Then, due to that ability, the RBD region only is fully enough to be used as the vaccine candidate so we do not have to use the entire protein sequence. Then, if let says that the chosen epitope is less immunogenic because it is not a immunodominant part or there is mutation, we could still use it in combinations with adjuvants which are able to increase immunogenicity by inducing strong innate immune responses that enable adaptive immunity. The value of the antigenicity test results for this epitope is 0.5922, with the predictions of toxicity and allergenicity being non-toxin and non-allergenic, respectively. In addition, this epitope has a percent identical (homologous) value to proteins in the human body which is only 68.75%. Moreover, the protein blast by the UniProt Website (https://www.uniprot.org/blast/) proved that this epitope has 0% identically to the human body protein. This protein blast test is proving enough that this epitope has less probability of causing autoimmune responses in the human body, at least in the in silico phase. Then, this epitope has a percentage value of protein sequence identity with various samples of the Delta variant virus in Indonesia as a result of a conservation analysis of 99.63%. Thus, the hypothesis was accepted because it was found that the epitope matched the criteria to be a vaccine candidate.

Advantages and Challenges of the Vaccine Candidate on the Future

According to Di Natale et al. (2020), the traditional vaccines (like the virus-based vaccines, either the weakened or inactivated viruses) are likely to have several disadvantages, like causing allergic and autoimmune reactions, having low stability, and needing to be stored at a cold temperature. To overcome these difficulties, a promising strategy and innovative approach may be the development of peptide-based vaccines. This study has provided a potential vaccine candidate that is often capable to overcome disadvantages encountered by other strategies by targeting very specific epitopes, removing the risks associated with allergic, and autoimmune responses. Moreover, it is more be a safe profile since its easiness of purification. Their chemical synthesis renders them suitable for large-scale production with low costs and high reproducibility. Normally, they are also soluble in water and more stable in storage conditions.[27-30]

Besides all of the advantages and strengths behind it, we considered this in silico research would be followed by the potential limitations. First, probably there would be another different result than what is written in this study according to the algorithm of the other website/application used. Second, this current study is limited to the predictions alone and ultimately needs an improvement to be confirmed experimentally by the in vitro research in the laboratory. Third, this study running based on the Delta variant, so it means that probably the results would be different if it is running by the other variants, either that already exists before or would be coming in the future. Finally, there would be another variant of the SARS-CoV-2 in the future, so there is also

Table 8. Selected peptides compare to the SARS-CoV-2 B.1.1.529 (Omicron) variant by the conservancy analysis

Selected peptides from the Delta variant	Comparison in the Omicron variant
QCVNLRTRTQLPPAY T	QLPPAY T
FQTLL ALHRS AAAYY	FQTLL ALHRSYSGWT AGAAAYY
FASTEKSNI IRGWIF	FASIEKSNI IRGWIF
PNITNLCPFG EVFNATRFAS	PNITNLCPFD EVFNATRFAS
VYAWNRKRIS NCVA	VYAWNRKRIS NCVA
FTNVYADS FVIRGDEVRQ	FTNVYADS FVIRGDEVRQ
IAPGQTGKIA DYNYKLPDDF T	IAPGQTGNIA DYNYKLPDDF T

another in silico study possibility (because of its mutation probability) for keeping up the peptides sequence to still be eligible for use as the vaccine candidate.

Furthermore, the results of the conservancy analysis mentioned above in Table 8 show the selected epitopes are also still promising to work against the omicron variant even there is some minor mutation in it. But, absolutely, there must be another continuous in-silico and in-vitro research to make sure that this peptide could truly work as the vaccine candidate against not only for the Delta but also for the Omicron or if there would probably be another future variant of SARS-CoV-2.

CONCLUSION

Based on in silico research that has been carried out, the predicted B-cell epitope sequence that can be used as a candidate for the SARS-CoV-2 B.1.617.2 (Delta) variant vaccine is FTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKL PDDFT. The predicted epitope is known as non-toxic, non-allergenic, and perhaps not causing any autoimmune responses in the human body. This selected epitope also has a percentage value of protein sequence identity with various samples of the SARS-CoV-2 B.1.617.2 (Delta) variant, either in Indonesia or overseas so it could be used as the vaccine candidate with a large scope. We considered that there are some high-risk mutations that probably would be on the future protein sequence of the SARS-CoV-2, especially on the prediction epitope that we have observed by this study. The mutation not only threatens the others variants but also the Delta itself. Therefore, there should be further development, more in-depth, and comprehensive in vitro research in the future to approve and improve this study. For the in silico research itself, we hope there would be another study with the different approaches for finding and developing vaccine candidates, not only for the SARS-CoV-2 but also for other viral infections.

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