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Research Article

Epitope Mapping of ORF3a Protein SARS-CoV-2 in Indonesia through Computational Study

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ABSTRACT

In Indonesia, the vaccine for SARS-CoV-2 is still being developed. ORF3a protein of SARS-CoV-2 could be a potential peptide for the vaccine, therapeutic antibodies, and diagnostic kit development. We used the computational approach in designing some potential epitopes against the ORF3a protein of the virus. The reference sequences of ORF3a SARS-CoV-2 were retrieved from GISAID. Our previous study found 203 non-synonymous mutations from 3,751 samples in Indonesia. We categorized and compared the variation in this protein with reference sequences. To predict B cell epitopes, we used Immune Epitope Database (IEDB) and VaxiJen v.2.0 as analysis resources. We found two epitopes, $_{177}$SPISEHDY$_{184}$ and $_{74}$SKGVHFVCNLLLLFVTVYSHLLLVAAG$_{100}$, that indicate antigen. Our study showed these predicted peptides could be used as a reference for the SARS-CoV-2 vaccine, therapeutic antibodies, and diagnostic development. This approach needs further studies to understand the functionality of the predicted proteins against SARS-CoV-2 that circulated, especially in Indonesia.

Keywords: Bioinformatics, Epitope, Mapping, ORF3a, SARS-CoV-2

Introduction

The pandemic cases of Coronavirus Diseases-19 (COVID 19) have not disappeared yet. Since the first outbreak at the end of 2019, this pandemic has spread worldwide and caused morbidity and mortality. According to the data from WHO, the number of confirmed cases had reached 260 and 5,2 million death cases worldwide per November 2021. Meanwhile, in Indonesia, the number of confirmed cases had reached 4.2 million cases and 143 billion deaths per November 2021 [1]. Moreover, a new variant, omicron, was developed in Indonesia and some countries. This variant phenomenon is common in the Coronavirus family because some mutations make amino acid changes in the viral protein. Such as the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), virus that has four main structural proteins, nucleocapsid (N), membrane (M), envelope (E), spike (S), and some nonstructural and accessory protein. This virus was undergoing continuous mutated certain genes from wild-type [2]. Researchers studied the genome of SARS-CoV-2 that is responsible for causing a going pandemic. The spike protein was used to develop a vaccine in CanSino Biological Inc. with Beijing Institute of Biotechnology, China, and the University of Oxford with AstraZeneca, etc. [3]. Some studies used nucleocapsid protein to develop a new vaccine [4, 5]. The utilization of spike protein showed in the study for therapeutic monoclonal antibodies; some of them had received emergency use authorization from the Food and Drug Administration (FDA) [6, 7]. Some proteins can be used for the development diagnostic kit, such as spike, envelope, nucleocapsid, and ORF1ab of SARS-CoV-2 with the various platform and manufacturers [8,9].

The efficacy of SARS-CoV-2 vaccines has been studied to know the immune response against several variants of concerns that could threaten public health. Some evidence showed a progressive decline in efficacy using the messenger RNA (mRNA) vaccine, such as mRNA BNT161b2

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(Pfizer-BioNTech) and mRNA-1273 (Moderna) [10-12]. A rapid decline in immune response occurred in inactivated SARS-CoV-2 vaccine (CoronaVac®, Sinovac) and ChAdOx1 nCoV-19 (Vaxzevria®, Ox-ford-AstraZeneca) [13]. In addition, several factors affected humoral response after the administration of mRNA vacci-nes, like age, sex, serostatus, and other comorbid [14]. Diagnostic concerns such as specimen processing problems, results interpretation, errors identifications, and cross contaminations would become an issue to meet the demand of many tests [15]. These findings show the necessity of developing vaccination strategies, especially to provide booster vaccines and overcome new variants. Furthermore, designing new and reliable diagnostic test kits to overcome cross-contamination in the lab was needed, especially in Indonesia's low-resource setting.

We used the ORF3a protein of SARS-CoV-2 to develop potential peptides for the vaccine, therapeutic, and diagnostic. The ORF3a are humoral immunogens in other coronaviruses and have a major role in virulence and infectivity, ion channel activity, and virus releases in SARS-CoV-2 [2]. The virus, which has a mutation at amino acid site 57 (Q→ H), leads to a major truncation and causes the fourth epidemic wave of COVID-19 in Hongkong and China. This mutation can make the virus evades the initiation of cytokine, chemokine, and interferon-stimulated gene [16]. Importantly, there was significant reactivity for ORF3a SARS-CoV-2 of the total CD4+ and CD8+ T cell responses [17].

In our previous study [18], we identified the 203 non-synonymous mutations of ORF3a in 3751 samples in Indonesia, compared with the reference sequence employed by GISAID (EPI_ISL_ 402124) [19]. For this study, we use a computational approach in designing some potential epitopes against the ORF3a protein of the virus based on the frequency of non-synonymous mutations. The frequency mutations help to determine the conserved domain for the epitope. Furthermore, the ORF3a protein is relatively conserved across fifteen various genomes of Coronavirus from three hosts (human, bat, and pangolin) [20]. The potential peptide from our result as a preliminary study could help the other researcher develop the vaccine, therapeutic antibodies, and diagnostic assay based on ORF3a according to Indonesian samples.

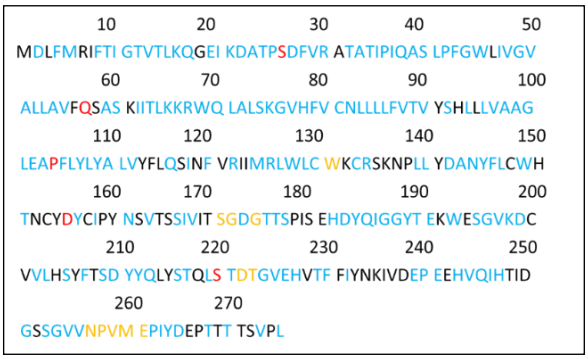

Figure 1. The ORF3a SARS-CoV-2 protein sequences. The mutated amino acids are shown in three colors according to percentages (blue <1%, yellow 1-5%, red> 5%), whereas the black letter was similar to reference sequences. The number indicates the amino acid position of ORF3a SARS-CoV-2.

Table 1. The predicted peptide of ORF3a SARS-CoV-2 with comparison mutated aminoacid and antigen score by VaxiJen

Peptide	Location	Sequences	Antigen Score (Tresh-old= 0.4)
1	74-100	SKGVHFVCNL LLLFVTVYSH LLLVAAG	0.5843*
2	177-184	SPISEHDY	0.5981*
3	198-206	KDCVVLHSY	0.1922
4	235-243	KIVDEPEEH	0.1057
5	262-271	PIYDEPTTTT	0.2550

*Probable antigen

Material and Methods

Data samples and mutations

We categorized 203 non-synonymous mutations of ORF3a SARS-CoV-2 protein from 3,751 samples in Indonesia from our previous study. These data were retrieved from the GISAID database. We conduct the multiple sequence alignment using BioEdit software with reference sequence hCoV-19/Wuhan/WIV04/2019 (EPI_ISL_402124).

B-cell epitope and discontinuous B-cell epitopes predictions

We predict the ORF3a SARS-CoV-2 epitope

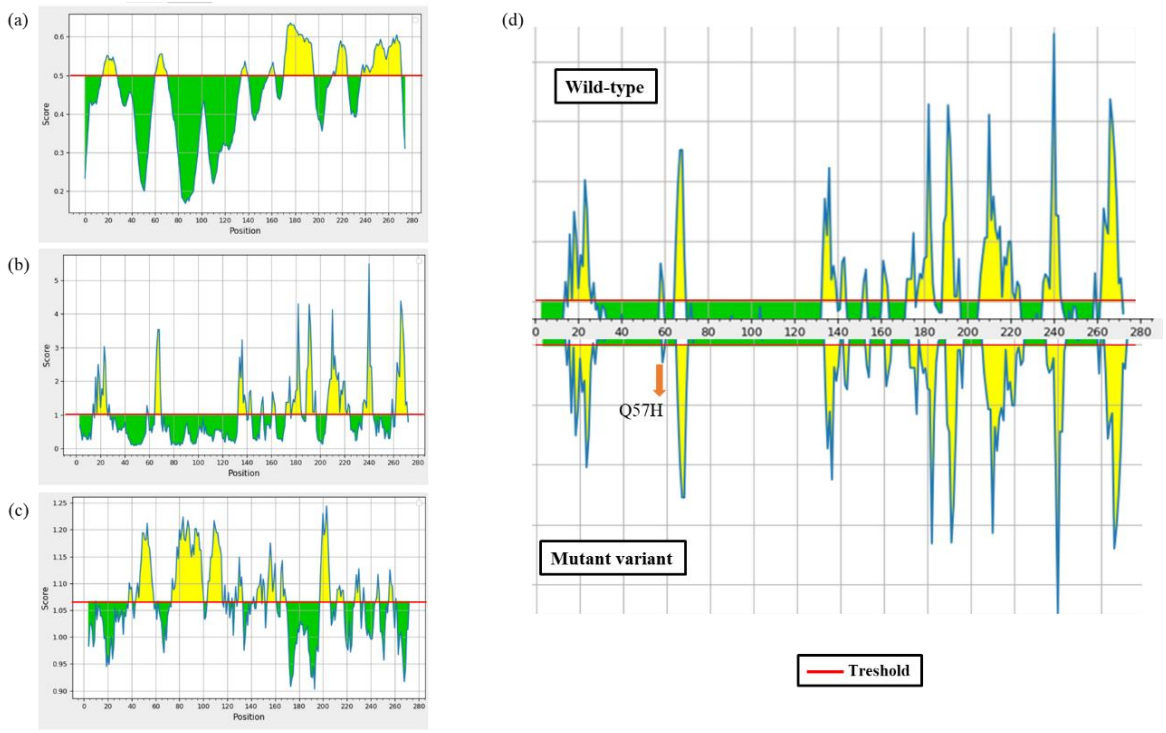

Figure 2. Prediction of B-cell epitope and antigenicity. (a) Bepipred linear epitope, (b) Emini surface accessibility, (c) Kolaskar and Tongaonkar Antigenicity, (d) Decreasing epitope at 57 amino acid residues of ORF3a SARS-CoV-2.

using IEDB Analysis tools. Three methods were chosen to predict the epitope: Bepipred linear epitope, Emini surface accessibility, Kolaskar, and Tongaonkar Antigenicity. We also predict the Discontinuous B-cell epitopes using Discotope - 2.0 in IEDB Analysis tools [21].

Epitope 3D-visualization

In this process, the five potential epitopes of B cells based on epitope mapping analysis were visualized using RasWin v2.7.5.2 software. We used PDB 6XDC [22].

Antigenicity test

The five potential epitopes were tested for their antigenicity using the VaxiJen v2.0 online tools [23]. From this test, only two out of five predicted peptides were considered to have antigenic features.

Results and Discussion

Amino acid mutations of ORF3a SARS-CoV-2

The ORF3a SARS-CoV-2 has 275 amino acids. We compare the 203 synonymous mutations with the reference sequence and give them a different color to show the percentage of mutations. This identification of mutations is looking for a conserved domain for peptide design. The mutated amino acids are shown in blue, yellow, and red colors with variations below 1%, 1-5%, and above 5%, respectively (Figure 1). The black color indicates no amino acid changes. Based on data, almost the amino acid in ORF3a has mutations below 1% (blue color). It shows the virus still develops new variations most likely to evade the immune system, thus causing viral transmission [24]. The non-synonymous mutations in ORF3a may less impact on the epitope variability than those found in nucleocapsid. Furthermore, ORF3a may indicate a more immunodominant role in intra-cellular processing responses than the nucleocapsid protein. Therefore, the ORF3a protein suggested in the current target for diagnostics and vaccination study potentially elicited the protective responses in protein and DNA forms [25].

Prediction of B-cell epitopes

The protein sequences for submission in B-cell epitope prediction were retrieved from protein consensus samples in Indonesia. These protein consensus sequences were conducted by looking for the dominant protein from multiple-sequence alignment, using 3,751 samples in our previous

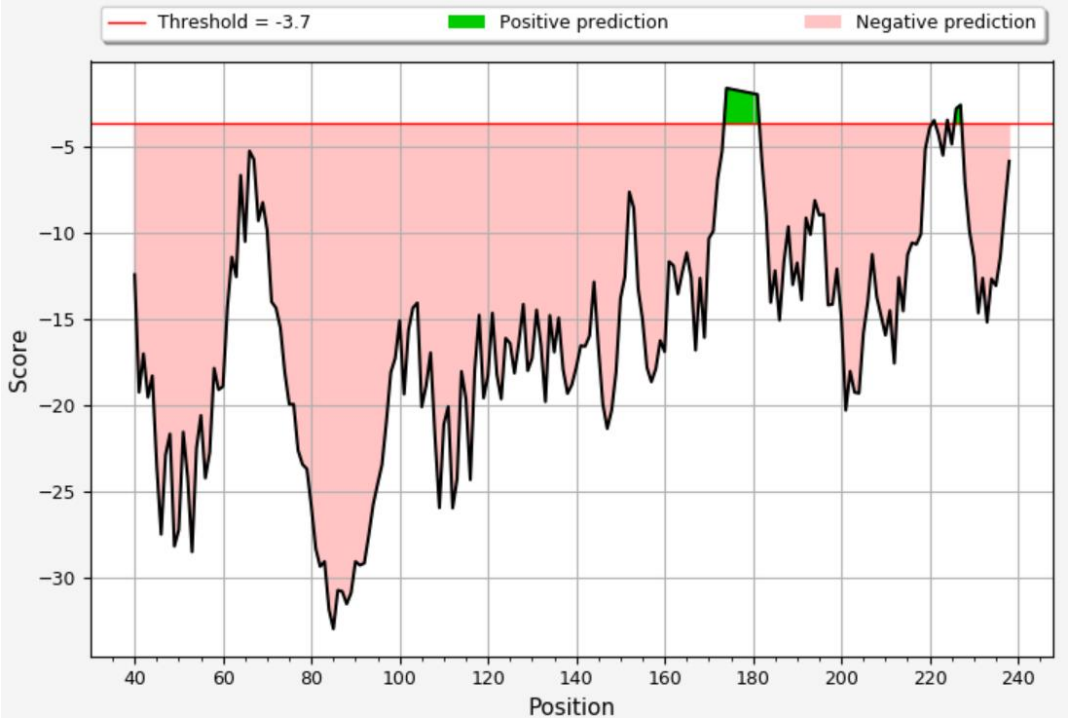

Figure 3. Prediction of discontinuous B-cell epitope. The green color indicates positive prediction while the pink color was a negative prediction

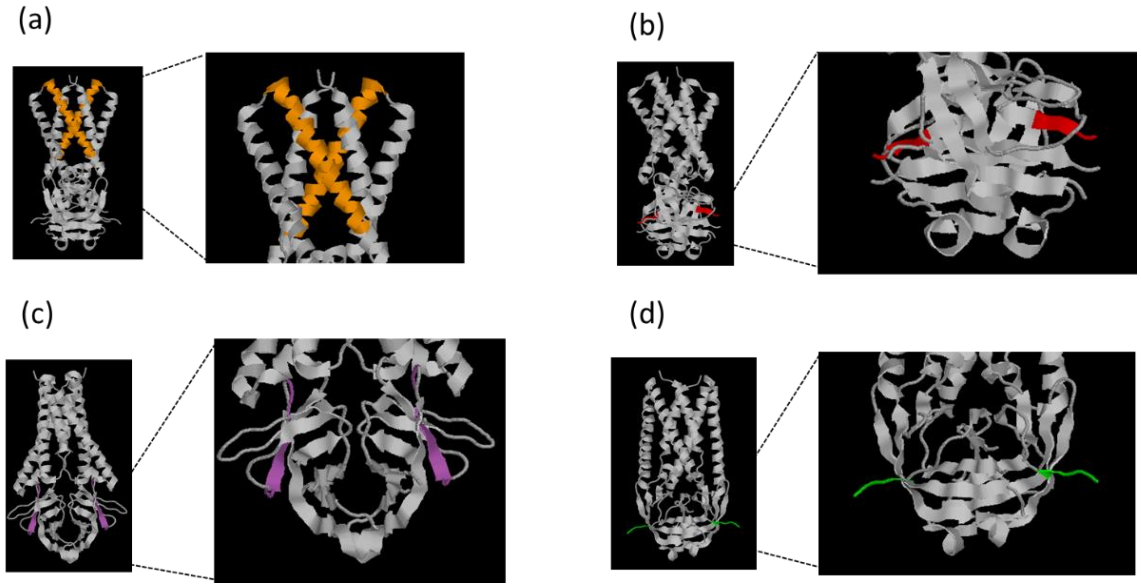

Figure 4. ORF3a of SARS-CoV-2 protein with four potential epitope of B cells. (a) 74-100; (b) 177-184; (c) 198-206; (d) 235-243.

study. We used some predictions on the web to optimize the purpose peptide. The yellow domain on the graphs might have the probability of being part of the epitope.

The first by Bepipred linear epitope prediction shows eight predicted peptides (Figure 2a). Emini surface accessibility prediction and Kolaskar and Tongaonkar Antigenicity show 7 and 6 predicted peptides, respectively (Figure 2b-c). We choose the peptide with the larger score for the residues (Y-axis) on the graphs. Furthermore, we identified the peptides with the mutations of ORF3a SARS-CoV-2 in this study. We exclude the peptide that consists of yellow and red colors based on the

identification of mutation results (Figure 1). According to the previous parameter, a total of 5 predicted peptides were obtained (Table 1). In addition, compared to wild-type epitope, we found a decreasing epitope in the mutant variant with amino acid changes in Q57H (Figure 2d). This phenomenon indicates that amino acid changes can lead to modification in the structure, such as loss or decreased epitope and might enhance the virus's antigenic diversity [26].

Prediction of discontinuous B-cell epitopes

The five predicted peptides use the linear epitope to analyze the data using B-cell epitope prediction tools before. We use the discontinuous B-cell epitopes to provide information about the effectivity predicted peptide in the conformational epitope. This prediction combines spatial properties and surface localization of the ORF3a SARS-CoV2 structure. Based on data, the amino acid in locations 174-181 shows positive predictions. This result means the predicted peptide $_{177}$SPISEHDY$_{184}$ might be effective in vaccine design or diagnostic tests that require conformational epitope (Figure 3).

Epitope 3D-visualization

The predicted peptides are then visualized in the ORF3a protein (Figure 4). We use the reference of ORF3a protein from Protein Data Bank with number ID PDB: 6XDC [27]. Based on the results, the location of the purpose peptide or epitope was exposed to the outside, and it is likely to be recognized by the immune system [28]. We could not visualize one peptide at position $_{262}$PIYDEPTTTT$_{271}$ because, in the protein data bank, the N-terminal and C-terminal were truncated.

Conclusion

This study showed predicted epitope has potential as a reference for the SARS-CoV-2 vaccine, therapeutic antibodies, and diagnostic development. We proposed two epitopes. These epitopes can exchange for placement, so the first for the long peptide and after that the short peptide. that indicate antigen by VaxiJen results. In addition, the epitope $_{177}$SPISEHDY$_{184}$ can use for the development assay in the conformational epitope. The predicted peptides need further study to know the interaction of peptides with the antibody receptor. In vitro and in vivo test is needed to determine the effectivity of proposed epitopes.

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