Vaccine Research
ISSN: 2423-4923
eISSN: 2383-2819

SARS-CoV-2 Lambda and Omicron Variants: Antigenicity Evaluation of Spike Proteins as Potential Targets for Vaccine Development

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ARTICLE INFO

Research Article

VacRes, 2023
Vol. 10, No.1, 1 - 5
Received: June 16, 2023
Accepted: July 09, 2023
Pasteur Institute of Iran

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KEYWORDS: SARS-CoV-2, lineage C.37, mutation, spike protein, Epitope, *In-silico*

ABSTRACT

Introduction: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread worldwide as an Omicron variant (B.1.1.529). Compared with the original SARS-CoV-2, this variant has more than 30 mutations on its spike. The Lambda variant (known as SARS-CoV-2 lineage C.37) is another variant of interest. The Lambda spike protein bears seven mutations; G75V, T76I, L452Q, F490S, D614G, T859N, and Δ247-253. The effect of such mutations on immune escape from neutralizing antibodies and infectivity is unknown. **Methods**: *In-silico* tools were applied to predict the antigenicity of the spikes of Lambda and Omicron variants and the results were compared to the reference Wuhan spike protein antigenicity. SWISS-MODEL, MolProbity, and QMEAN were used for model quality assessment. DiscoTope2.0, BEpro, and Ellipro were used for the prediction of conformational and linear B cell epitopes. **Results**: The evaluation of the obtained modeled proteins showed that the predicted models by Swiss-Model had higher quality for the Lambda and Omicron spikes with 0.56% and 1.63% of residues in outlier and 94.39% and 92.51% residues in favored regions, respectively. The results of conformational B cell epitope prediction showed 6 epitopic regions on S1 of Lambda spike and 1 epitopic region on the S2 segment of the protein. For the Omicron variant, 9 epitopic regions existed on S1 and 1 epitopic region (1137-1159) was on S2. **Conclusion**: Our results suggested that B cell epitope removal and reducing the antigenicity properties of the epitopic residues involve reducing susceptibility to antibody neutralization of the mutant protein.

INTRODUCTION

The coronavirus outbreak continues to evolve, as observed by Severe Acute Respiratory Syndrome-CoV (SARS-CoV) in 2003, Middle East Respiratory Syndrome-CoV (MERS-CoV) in 2012, and a novel coronavirus named "2019 novel coronavirus (2019-nCoV)" which emerged in late December 2019 in Wuhan, China [1]. The International Virus Classification Commission (ICTV) designated 2019-nCoV as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Since the emergence of SARS-CoV-2, new lineages of this virus have been described. The recent emergence of SARS-CoV-2 variants of interest (VOI) and variants of concern (VOC) with reduced sensitivity to antibody neutralization and possibly increased transmissibility, are considered to be potential threats to the public health. The genome organization and expression of all CoVs are similar, in which open reading frame (ORF) 1a/b at the 5′ end encodes 16 nonstructural proteins (*i.e.*, nsp1-16), followed by the structural proteins, namely, spike (S), envelope (E), membrane (M), and nucleocapsid (N), which are encoded by other ORFs at the 3′ end [2].

A specific monoclonal antibody (mAb) can target the S protein of SARS-CoV which is a transmembrane glycoprotein that allows the viral entry to human respiratory epithelial cells by interacting with cell surface receptor angiotensin-converting enzyme 2 (ACE2) [3]. A 193 amino acid length fragment within the S protein, receptor-binding domain (RBD), contains the critical neutralizing antibodies target [4]. The RBD within the S protein of SARS-CoV-2 interacts with ACE2 [5, 6] and has shown a high-affinity for binding with SARS-CoV-specific neutralizing antibodies, CR3022 [7]. The Lambda variant (within B.1.1.1 lineage, termed C.37) is another VOI (designated on June

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[DOI: 10.61186/vacres.10.1.1]

14, 2021) [8]. The presence of this variant has been reported in 26 countries as of June 2021 with most of the available sequences coming from South American countries, particularly Chile, Peru, Ecuador, and Argentina. This variant picked up multiple changes (substitutions and deletions) in the spike protein including G75V, T76I, L452Q, F490S, D614G, T859N, and Δ247-253 [9]. SARS-CoV-2 consequently spread worldwide as an Omicron variant (B.1.1.529). This was a heavily mutated variant virus and was designated as a variant of concern by the World Health Organization (WHO).

Computational methods can predict how specific mutations in the receptor binding domain (RBD) of SARS-CoV-2 affect the virus's binding affinity to the ACE2 receptor. This approach involves the use of bioinformatics tools and molecular modeling techniques to provide insights into virus evolution, transmission, and potential targets for drug or vaccine development. Several studies have utilized computational methods to investigate the effects of RBD mutations on SARS-CoV-2 binding to ACE2 [10-12]. For instance, Wang et al. utilized molecular dynamics simulations to study the impact of RBD mutations found in the Alpha (B.1.1.7) and Beta (B.1.351) variants on ACE2 binding. Their results showed that these mutations decreased the binding energy between SARS-CoV-2 RBD and ACE2, which may affect viral entry and immune recognition [10]. Another study utilized computational modeling to predict the potential impact of RBD mutations on the efficacy of monoclonal antibodies against SARS-CoV-2. The authors found that mutations in the RBD could affect the binding of several monoclonal antibodies, including bamlanivimab and casirivimab, which obtained emergency use authorization in the United States [11]. Moreover, machine learning algorithms were used to predict the impact of RBD mutations on the transmissibility of SARS-CoV-2, and the authors found that mutations in the RBD, particularly those affecting the electrostatic potential and solvent accessibility of the protein, might contribute to the viral transmission and adaptation [12].

In this study, we applied homology modeling to build S protein structures of Lambda and Omicron variants. Further, the antigenicity of the spike of these variants was predicted and compared to the reference Wuhan spike protein antigenicity.

MATERIALS AND METHODS

In-silico Methods

To identify key mutations in emerging SARS-CoV-2 variants that may affect the viral entry and immune evasion, firstly, the sequence of the reference Wuhan spike protein was retrieved from the Uniprot Knowledge Base. The mutations specific to the variants were introduced into the sequence and predicted the 3D structure of the mutated S protein using homology modeling by SWISS-MODEL server. The accuracy of the predicted models was evaluated using MolProbity and QMEAN servers. Next, the conformational and linear B-cell epitopes of the modeled protein structures were predicted using three different servers, namely, DiscoTope2.0, BEpro, and Ellipro. The epitopes that predicted by the three servers were selected as epitopes, and their antigenicities were compared to the reference Wuhan spike protein antigenicity (Fig. 1).

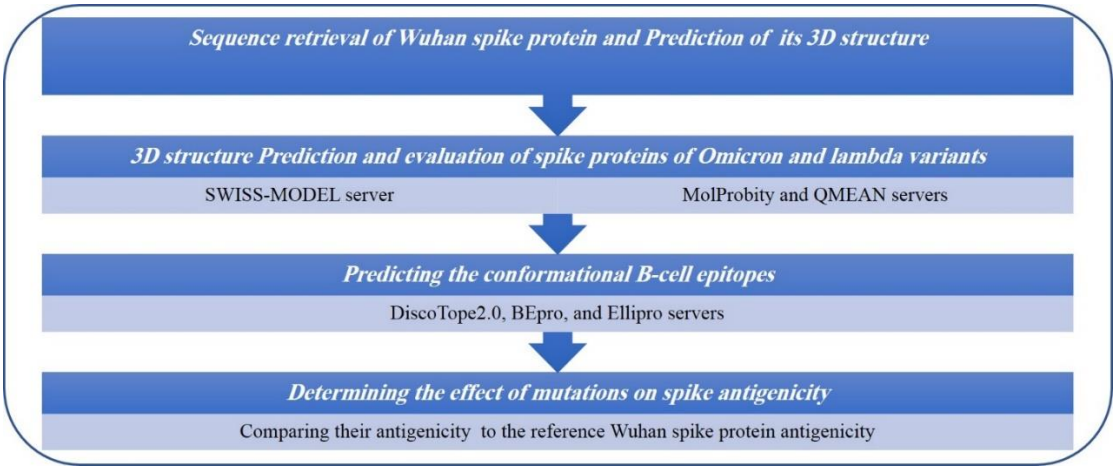

Fig. 1. A flowchart of the performed investigations.

Sequence Retrieval and 3D Structure Prediction and Evaluation

The sequence of the spike protein was obtained from the Uniprot Knowledge Base (UniprotKB) [13] at <www.uniprot.org> (accession number: P0DTC2). The structural prediction of mutated S protein was done by SWISS-MODEL homology modeling server [14] (https://swissmodel.expasy.org/. The 3D structure of the reference spike protein was available; however with high missing residues. Therefore, modeling of this protein was performed to refine their structures. The predicted models were evaluated by MolProbity [15] and QMEAN <https://swissmodel.expasy.org/qmean/> [16] servers.

Linear and Conformational B cell Epitopes Prediction

The conformational and linear B cell epitopes of the modeled protein structures were predicted through three servers, namely DiscoTope2.0, BEpro, and Ellipro. DiscoTope2.0 tool [17] <http://www.cbs.dtu.dk/services/DiscoTope/> predicts through combining an epitope propensity amino acid score and the surface accessibility (estimated regarding contact numbers).

A score threshold of (-3.7) was used for epitope prediction. BEpro (previously known as PEPITO) [18] invokes a combination of amino-acid propensity scores and half-sphere exposure values at multiple distances to predict. Residues scored ≥ 1 were considered as conformational B-cell epitopes. Ellipro

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predicts based on the geometrical properties of protein structure with an AUC value of 0.732 [19]. The residues that predicted by three servers as an epitope were selected as epitopes.

RESULTS

Structural Prediction and Evaluation

Evaluation of the obtained modeled proteins showed that the predicted 3D models by Swiss-Model had higher quality for Lambda and Omicron spikes with 0.56% and 1.63% of residues in outlier and 94.39% and 92.51% residues in favored regions, respectively. The best models were tri-mer protein with QmeanDisCo of 0.75 ±0.05 and 0.72 ±0.05 for Lambda and Omicron, respectively. The best model of the reference protein had high quality with 1.21% and 94.45% of residues in outlier and favored regions, respectively. The QmeanDisCo of the best model was 0.74 ±0.05. The detailed quality information on the best model of the proteins is shown in Table 1.

Table 1. Model quality scores of the spike proteins in different variants.

Spike protein of Lambda variant	Protein Geometry	Poor rotamers	61	2.05%	Goal: <0.3%
		Favored rotamers	2787	93.46%	Goal: >98%
		Ramachandran outliers	19	0.56%	Goal: <0.05%
		Ramachandran favored	3228	94.39%	Goal: >98%
		Rama distribution Z-score	-0.76 ± 0.14		Goal: abs(Z score) < 2
		Cβ deviations >0.25Å	53	1.65%	Goal: 0
		Bad bonds:	8 / 27399	0.03%	Goal: 0%
		Bad angles:	203 / 37293	0.54%	Goal: <0.1%
	Peptide Omegas	Cis Prolines:	0 / 162	0.00%	Expected: ≤1 per chain, or ≤5%
		Twisted Peptides:	1 / 3423	0.03%	Goal: 0
	Low-resolution Criteria	CaBLAM outliers	90	2.6%	Goal: <1.0%
		CA Geometry outliers	32	0.94%	Goal: <0.5%
	Additional validations	Tetrahedral geometry outliers	2		
Spike protein of SARS-CoV-2	Protein Geometry	Poor rotamers	61	2.05%	Goal: <0.3%
		Favored rotamers	2787	93.46%	Goal: >98%
		Ramachandran outliers	19	0.56%	Goal: <0.05%
		Ramachandran favored	3228	94.39%	Goal: >98%
		Rama distribution Z-score	-0.76 ± 0.14		Goal: abs(Z score) < 2
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	Peptide Omegas	Cis Prolines:	0 / 162	0.00%	Expected: ≤1 per chain, or ≤5%
		Twisted Peptides:	1 / 3423	0.03%	Goal: 0
	Low-resolution Criteria	CaBLAM outliers	90	2.6%	Goal: <1.0%
		CA Geometry outliers	32	0.94%	Goal: <0.5%
	Additional validations	Tetrahedral geometry outliers	2		
Spike protein of Omicron variant	Protein Geometry	Poor rotamers	61	2.05%	Goal: <0.3%
		Favored rotamers	2787	93.46%	Goal: >98%
		Ramachandran outliers	19	0.56%	Goal: <0.05%
		Ramachandran favored	3228	94.39%	Goal: >98%
		Rama distribution Z-score	-0.76 ± 0.14		Goal: abs(Z score) < 2
		Cβ deviations >0.25Å	53	1.65%	Goal: 0
		Bad bonds:	8 / 27399	0.03%	Goal: 0%
		Bad angles:	203 / 37293	0.54%	Goal: <0.1%
	Peptide Omegas	Cis Prolines:	0 / 162	0.00%	Expected: ≤1 per chain, or ≤5%
		Twisted Peptides:	1 / 3423	0.03%	Goal: 0
	Low-resolution Criteria	CaBLAM outliers	90	2.6%	Goal: <1.0%
		CA Geometry outliers	32	0.94%	Goal: <0.5%
	Additional validations	Tetrahedral geometry outliers	2		

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Prediction of Linear and Conformational B cell Epitopes

The results of conformational B cell epitope prediction (Table 2) showed 6 epitopic regions on S1 of Lambda spike including 146-151, 182-184, 436-443, 485-487, 489-498, and 671-681 and 1 epitopic region, 1136-1155, on the S2 segment of the protein. For Omicron variant, 9 epitopic regions (72-74, 143-147, 175-180, 246-250, 441-446, 455-459, 495-502, 675-685, 806-809) existed on S1 and 1 epitopic region (1137-1159) on S2. To determine the effects of the mutations on spike antigenicity, the antigenicities of the mutated proteins were compared with the reference protein (Fig. 2).

Table 2. Conformational epitopes on spike protein of Lambda and Omicron variants.

Variant	Epitopes
Lambda	72(G), 74(N), 146-151(HKNNKS), 182-184(KQG), 408-409(TG), 433(N), 436-443(SKVGGNYN), 476(V), 485-487(LQS), 489-498(GFQPTNGVGY), 671-681(TNSPRRARSVA), 802-803(PS), 805(P), 1093(T), 1136-1155(PELDSFKEELDKYFKNHTSP)
Omicron	72-74(NGT), 143-147(NNKSW), 175-180(EGKQGN), P204, I206, E208, 246-250(LTPGD), 441-446(KVSGNY), 455-459(KSNLK), P476, S493, 495-502(RPTYGVGH), 675-685(TKSHRRARSVA), I791, 806-809(PSKP), 1137-1159(PLQPELDSFKEELDKYFKNHTSP)

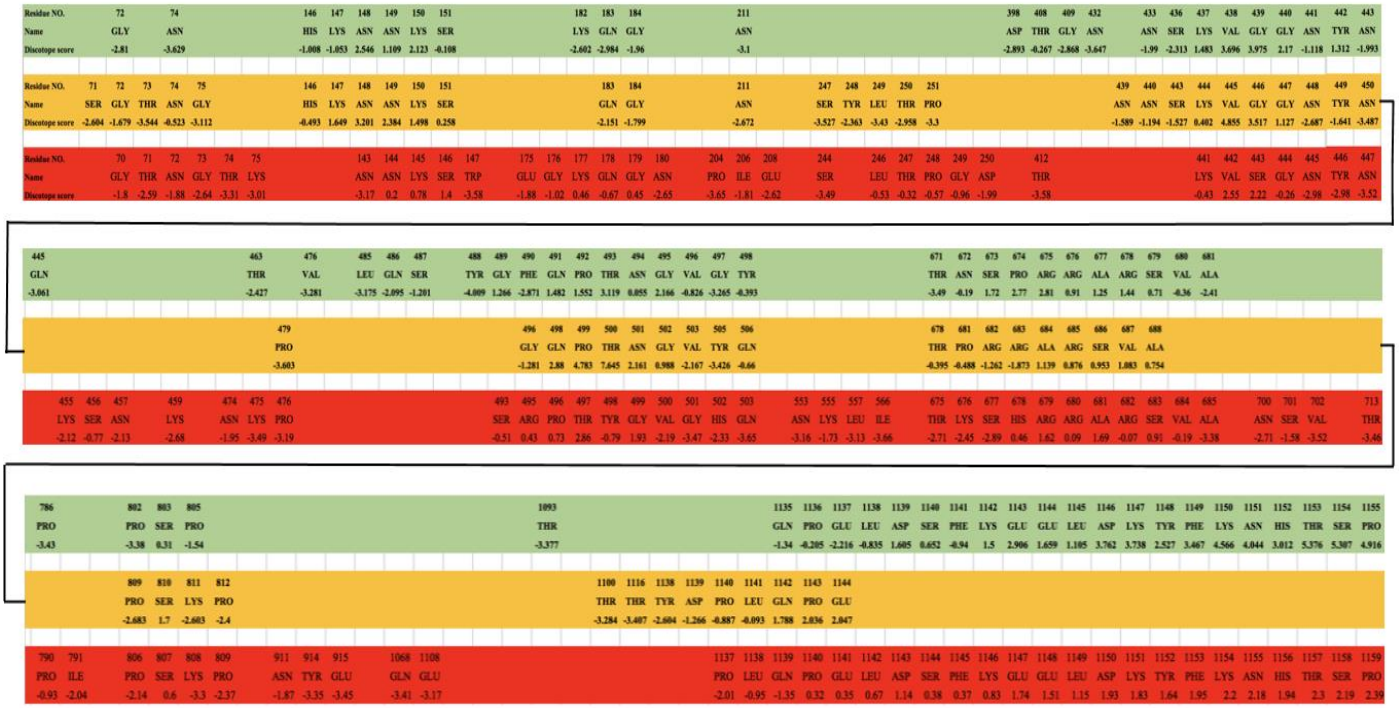

Fig. 2. Discotope score for the epitopic residues of the mutant spike protein of Lambda and Omicron variants compared with the reference (*i.e.*, Wuhan spike protein). The spike proteins of Lambda, Omicron and the reference are shown by green, red and yellow colors, respectively.

DISCUSSION

The Omicron variant has more than 30 mutations on its spike, including A67V, Δ69-70, T95I, G142D/Δ143-145, Δ211/L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493K, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F and The Lambda spike protein bears seven mutations; G75V, T76I, L452Q, F490S, D614G, T859N, and Δ247-253. These mutations have been associated with reduced susceptibility to antibody neutralization. The G75V and T76I mutations occur at an exposed loop in Lambda N-Terminal Domain (NTD). The Δ247-253 mutation also occurs at a loop (246 to 260) in Lambda NTD, which is a binding site for 4A8 MAb to the virus [20]. Interestingly, our obtained results as shown in Fig. 1 and Table 2, indicated that 71-75 and 146-151 epitopic regions are shortened and had reduced antigenicity score. While 247-251 epitopic region was not considered as an epitope in the mutant protein, L452Q and F490S mutations occur in RBD. Two predicted epitopes on RBD (i.e., 439-458, and 496-506) which bind to P2B-2F6 [21] and B38 [22] neutralizing antibodies, respectively, showed reduced antigenicity scores too. However, 677-688 epitopic region is longer and represents higher antigenicity scores for more residues. Two epitopes, namely 398-432 and 485-487 which were predicted in the mutant protein were not observed in the reference protein. Moreover, 802-805 epitope in the S2 segment showed reduced antigenicity.

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In the case of Omicron protein, two epitopic regions in the NTD segment (72-74 and 143-147) did not show reduced antigenicity and had shortened epitopic regions. The 246-250 epitopic region showed higher antigenicity than the reference protein. Two epitopic regions in RBD (439-458 and 496-506) showed decreased antigenicity scores; however, a few new epitopic regions (455-457,459, 474-476, 553,555,557 and 566) were also in this segment. Meanwhile, 677-688 epitopic region showed a longer epitopic region with higher antigenicity than the reference protein. In the S2 segment, 809-812 epitopic region also showed a reduced antigenicity score while some new epitopic regions were also observed in this segment.

Finally, our results suggested that B cell epitope removal and reduced antigenicity properties of the epitopic residues are involved in reducing the susceptibility to antibody neutralization of the mutant protein. It is envisaged these observations would help the researchers to understand the possible effects of the adapted mutations in the antigenicity of the spike protein of SARS-CoV-2 for potential prediction of efficient medications and vaccines against COVID-19.

ACKNOWLEDGEMENT

The authors wish to thank Shahid Rajaee Teacher Training University and Shiraz University of Medical Sciences for supporting the conduct of this research.

CONFLICT OF INTEREST

The authors declare they have no conflict of interests.

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