



Mutation-Induced Changes in the Stability, B-Cell Epitope, and Antigenicity of the Sars-Cov-2 Variant Spike Protein: A Comparative Computational Stud

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Keywords

Antigenicity; B-cell epitope; Mutation; SARS-CoV-2; S Protein

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RESEARCH PAPER

Mutation-induced Changes in the Stability, B-cell Epitope, and Antigenicity of the Sars-Cov-2 Variant Spike Protein: A Comparative Computational Study

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Abstract

The spike (S) protein is a major antigenicity site that targets neutralizing antibodies and drugs. The growing number of S protein mutations has become a severe problem for developing effective vaccines. Here, we investigated four severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants that were the most infectious and widespread during the COVID-19 pandemic to determine the trends and patterns of mutation-induced changes in the stability, B-cell epitope, and antigenicity of the SARS-CoV-2 S protein. The data showed that the Beta and Gamma variants had three mutations on the receptor-binding domain (RBD), which is the specific site on the S protein for angiotensin-converting enzyme 2 (hACE2) binding. The Delta variant had only two mutations, whereas the Omicron variant had 15 mutations on the RBD. The results showed that the stability of the S protein varied and depended on the mutation type and that Gamma and Omicron are the most stable of the four variants analyzed. The S protein–hACE2 complexes of the Beta and Gamma variants were relatively stable after 20 ns of simulation compared with those of the Delta and Omicron variants. We predicted that the B-cell epitopes of the mutant S protein would be different from those of the wildtype. Moreover, the antigenicity of Omicron changed drastically compared with that of the other variants. Bioinformatics analysis and a molecular dynamic simulation revealed that the mutations affected the stability of the S protein. A large number of mutations do not always stabilize the S protein. Mutations in Omicron significantly altered the B-cell epitope and antigenicity, which decreased vaccine effectiveness. These findings provide insights into SARS-CoV-2 evolution for vaccine development.

Keywords: Antigenicity, B-cell epitope, Mutation, SARS-CoV-2, S protein

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of the coronavirus disease (COVID-19), is a highly mutational RNA virus, as it lacks a proofreading mechanism. This virus continuously undergoes deletion, insertion, and genetic recombination that change critical amino acids in its genome. These amino acid changes benefit adaptation and evolution of the coronavirus. This continual series of mutations has led to a vast diversity of variants of the virus from

the original Wuhan sequence [1–3]. In February 2022, several variants of concern, including Gamma (P.1), Beta (B.1.351), Omicron (B.1.529), and Delta (B.1.617), were identified. The continuously mutating SARS-CoV-2 spike (S) protein is a severe problem for developing effective vaccines against COVID-19.

The S protein is a part of the structure that plays a unique and crucial function during the early stage of infection to angiotensin-converting enzyme 2 (hACE2), the target-cell host receptor. As a target of antibodies and drugs, the S protein is a major

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antigenicity site. Mutations in the S protein have become the primary concern of many studies because the S protein is an important target for neutralizing antibodies [4,5]. Each variant demonstrates a different mutation on its S protein. Mutations affect the stability and binding expression of S–hACE2; however, no study has compared the mutational effect of the SARS-CoV-2 S protein variants of concern. Therefore, in this study, we investigated four SARS-CoV-2 variants that were the most infectious and widespread during the pandemic (Beta, Delta, Gamma, and Omicron) to detect trends and patterns of how mutations in the S protein affect interactions with ACE2 due to structural and functional changes in the S protein. We also compared the effect of mutations of the B-cell epitopes and antigenicity of the four SARS-CoV-2 variants.

In this study, we investigated the mutational effect, epitope, and antigenicity of the four above-mentioned SARS-CoV-2 variants using bioinformatics analysis and molecular dynamic (MD) simulation and found that the stability of the S protein was affected by the mutations. The results of the MD simulations showed that Beta and Gamma were relatively more stable than Delta and Omicron, revealing that an increase in the number of mutations does not always correlate with stabilization of the S protein. Accumulated mutations on Omicron significantly changed its B-cell epitope and antigenicity, thereby decreasing the effectiveness of vaccines. These findings provide a more comprehensive understanding of SARS-CoV-2 adaptation and evolution.

2. Materials and methods

2.1. Data mining

The S–hACE2 crystalline structure was obtained from the Protein Data Bank (<http://www.pdb.org>). The

entire genome of the SARS-CoV-2 spike glycoprotein structure was constructed using the Swiss Model Homolog on the ExPASy web server (swissmodel.expasy.org). Sequences were retrieved from all selected variants of the protein using Biovia Discovery Studio.

2.2. Mutation collection

We introduced a mutation to the whole genome monomer S protein and the S protein complex, containing hACE2, by substituting amino acids based on the mutation list retrieved from the literature [10–12] to obtain the desired SARS-CoV-2 variants (Beta, Gamma, Delta, and Omicron) using the FOLDX5 plugin on YASARA (viralzone.expasy.org). Due to application limitations, the mutations introduced were limited to amino acid substitutions (i.e., no deletions or insertions). Amino acid substitutions were introduced into the S protein monomer throughout the genome, not just on the receptor-binding domain (RBD). The mutations were only introduced on the RBD of the S protein–hACE2 complex.

2.3. Protein stability calculation

The stability of the SARS-CoV-2 S protein and the S–hACE2 protein complex was predicted by the folding free energy change ($\Delta\Delta G$) between the wild-type (WT) and the mutant S protein structure. FoldX5 is a popular tool to assess the effect of a mutation on the folding and dynamics of nucleic acids and proteins [6].

The folding free energy change was calculated as follows [7]:

$$\Delta\Delta G (\text{stability}) = \Delta G (\text{folding})_{\text{mutant}} - \Delta G (\text{folding})_{\text{WT}}$$

A negative G value indicates that the mutation stabilizes the protein, whereas a positive G value indicates that the mutation destabilizes the protein.

Table 1. Mutation sites in the RBD S protein.

Origin	First Reported	Lineage	Mutation on S Protein	Ref
Beta (South Africa)	September 2020	B.1.351	K417N; E484K; N501Y; D614G; A701V	[10]
Gamma (Japan & Brazil)	January 2021	P.1	L18F; T20N; P26S; D138Y; R190S; K417T; E484K; N501Y; D614G; H655Y; T1027I	[10]
Delta (India)	October 2021	B.1.617	T19R, G142D, Δ 156–157, R158G, Δ 213–214, L452R, T478K, D614G, P681R, D950N	[11]
Omicron (South Africa)	November 2021	B.1.529	A67V; Δ 69–70del; T95I; G142D; Δ 143del, G143D, Δ 144–145del, Δ 211del; L212I; ins214EPE; G339D; S371L; S373P; S375F; K417N; N440K; G446S; S477N; T478K; E484A; Q493R; G496S; Q498R; N501Y; Y505H; T547K; D614G; H655Y; N679K; P681H; N764K; D796Y; N856K; Q954H; N969K; L981F	[12]

2.4. Molecular dynamics simulation

The effect of each mutation on the S protein was assessed, and the structural stability was predicted using MD. The MD analysis was based on the analysis of the physical movement of atoms and molecules during a simulation. The MD analysis was performed using YASARA with 20 ns of simulation time. The MD simulations were conducted on parameters that were adapted to cellular conditions. The system was neutralized with 0.9% NaCl and 1 atm of pressure while maintaining a pH of 7.4. The temperature was set to 298 K. The simulation was conducted with the md_runfast program in the autosave setting every 25 ps for up to 800 simulations.

2.5. Predicting the B-cell epitopes and antigenicity

The B-cell epitope was predicted using BepiPred-1.0 Linear Epitope Prediction at the iedb.org website. BepiPred is a machine learning-based method that predicts epitopes using the propensity scale and hidden Markov models [6,8]. The WT and SARS-CoV-2 mutant S protein antigenicity was predicted at the iedb.org website using the Kolaskar and Tongaonkar antigenicity scales [9].

3. Results and discussion

The SARS-CoV-2 variants exhibited different mutations on the S protein. Among the four variants analyzed, the Beta variant had the fewest mutations, with three mutations on the RBD and three mutations on the S sequence. The Gamma variant had 3 mutations on the RBD and 11 on the S sequence. The Delta variant had two mutations on the RBD and seven on the S sequence. The Omicron variant had 15 mutations on the RBD and 30 on the S protein (Table 1).

3.1. Mutations that altered the S protein structure and increased the protein stability

The S protein is important during the initial stages of virus infection. The S protein binds with hACE2 to enter the cell. Due to a strong correlation between structure and function, the structural stability of all mutant S proteins was analyzed to understand the effect of the mutation on protein stability. The S protein was stabilized after one mutation (Table 2), and the mutants had different degrees of stability depending on the mutation. Gamma and Omicron were the most stable variants.

The root-mean-square deviation (RMSD) backbone of the four SARS-CoV-2 variants showed the effect of mutation on the structural stability of the

Table 2. Amino acid sequence changes in the wildtype and SARS-CoV-2 mutant.

Variant	Position of Amino Acid										ΔG		ΔΔG											
	18	19	20	26	67	95	138	142	158	190	212	339		371	373	375	417	440	446	452	477	478	wildtype	mutant
Wildtype	L	T	T	P	A	T	D	G	R	R	L	G	S	S	S	K	N	G	L	S	T	750.06	749.52	-0.54
Beta	L	T	T	P	A	T	D	G	R	R	L	G	S	S	S	N	N	G	L	S	T	736.73	731.30	-5.43
Gamma	F	T	N	S	A	T	Y	G	R	S	L	G	S	S	S	T	N	G	L	S	T	751.34	750.84	-0.50
Delta	L	R	T	P	A	T	D	G	G	R	L	G	S	S	S	K	N	G	R	S	T	707.44	704.21	-3.23
Omicron	L	T	T	P	V	I	D	D	R	R	I	D	L	P	F	N	K	S	L	N	K	1027		
Wildtype	E	Q	G	Q	N	Y	T	D	H	N	P	A	N	D	N	D	Q	N	L	T	T	750.06	749.52	-0.54
Beta	K	Q	G	Q	Y	Y	T	G	H	N	P	V	N	D	N	D	Q	N	L	T	T	736.73	731.30	-5.43
Gamma	K	Q	G	Q	Y	Y	T	G	Y	N	P	A	N	D	N	D	Q	N	L	I	T	751.34	750.84	-0.50
Delta	E	Q	G	Q	N	Y	T	G	H	N	R	A	N	D	N	N	Q	N	L	T	T	707.44	704.21	-3.23
Omicron	A	R	S	R	Y	H	K	G	Y	K	H	A	K	Y	K	D	H	K	F	T	T			

S protein. The RMSD analysis indicated that the WT and mutants had high RMSD values after 20 ns of simulation time, which may have been due to an unstable S protein monomer. To be stable, the S protein must be in the trimer state. The stability of the variants was compared with that of the WT for the structural stability analysis (Fig. 1).

The fluctuating trends were not different among the WT, Gamma, and Delta variants. Omicron had a lower RMSD value despite its instability. The RMSD analysis of Beta indicated relatively stable fluctuations, starting at 13 ns of simulation. Beta was more stable than the other variants.

The root-mean-square fluctuation (RMSF) depicts the flexibility of the amino acid residue under study over the simulation time [13]. The RMSF analysis showed that the WT and mutants had similar fluctuating trends in the protein residues. The protein fluctuated the least during the start of the simulation, increased toward the middle, and then decreased at the end. In contrast, Gamma displayed the greatest fluctuations in the region between Phe392 and Thr581, the region between Phe833 and Lys854, and the site between Leu959 and Leu1001 compared with the other strains. Beta displayed the least fluctuations compared with the other strains in the same region. This RMSF result supports the RMSD data, showing that the amino acids in a

protein structurally contribute most to molecular motion.

Fig. 2 depicts the mutations in the S1-RBD of the SARS-CoV-2 variants. Beta and Gamma had similar mutations in the S1-RBD. The amino acids on the RBD of Beta and Gamma changed to E484K, N501Y, and K417N. The Delta variant only carried the L452R and T478K mutations. Omicron has recently evolved into many subvariants and has gained 15 mutations in its RBD.

The RMSD backbone of the S protein–hACE2 WT complex and mutant SARS-CoV-2 was also analyzed. The RMSD analysis revealed that Beta and Gamma had 2 Å values and relatively stable fluctuations after 20 ns of simulation, indicating that the S proteins of these variants were stable after binding to hACE2. Delta and Omicron had values exceeding 3 Å and were relatively unstable after 3 and 8 ns of simulation, indicating that the mutations disrupted the binding interactions between the S protein and hACE2.

3.2. Omicron antigenicity was altered drastically compared with the other variants

The B-cell epitope refers to the part of the antigen that binds to the antibody. The B-cell epitope is recognized by B-cell receptors (BCRs) and induces an antibody response [6]. Because the S protein

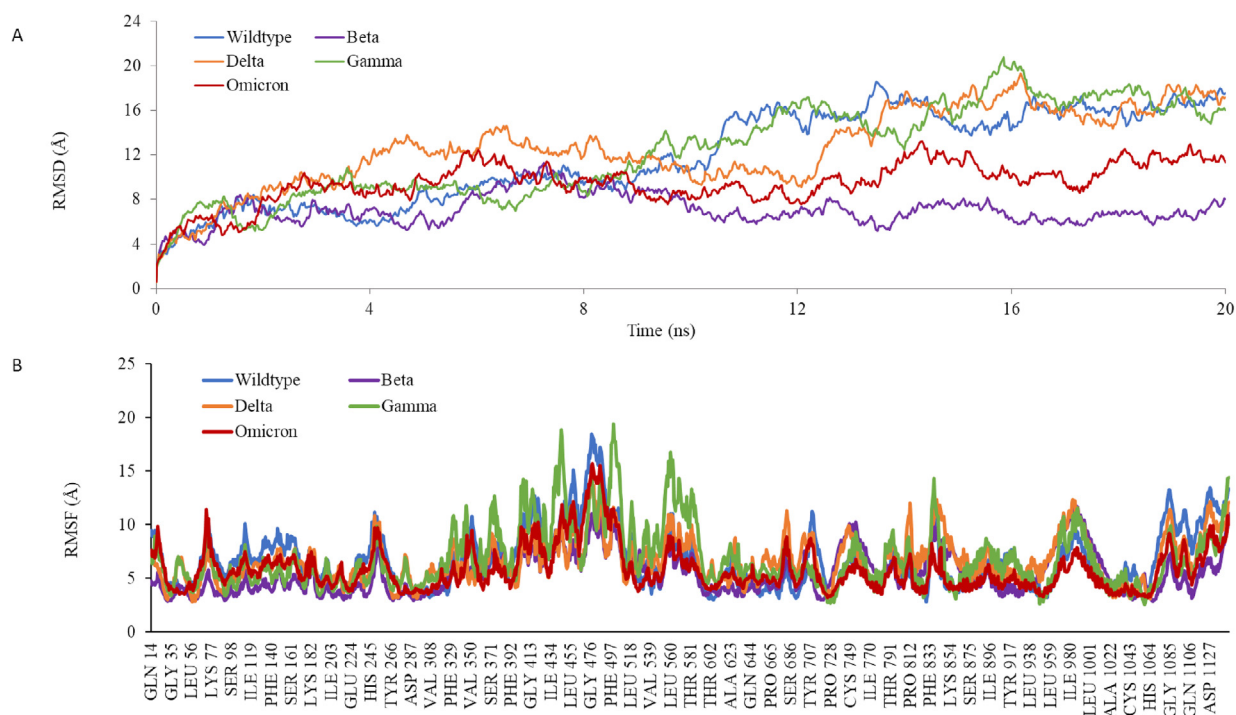


Fig. 1. Analysis of trajectory fluctuations in the molecular dynamics simulations of the SARS-CoV-2 wildtype and mutant S proteins. (A) Root-mean-square-deviation. (B) Root-mean-square fluctuation.

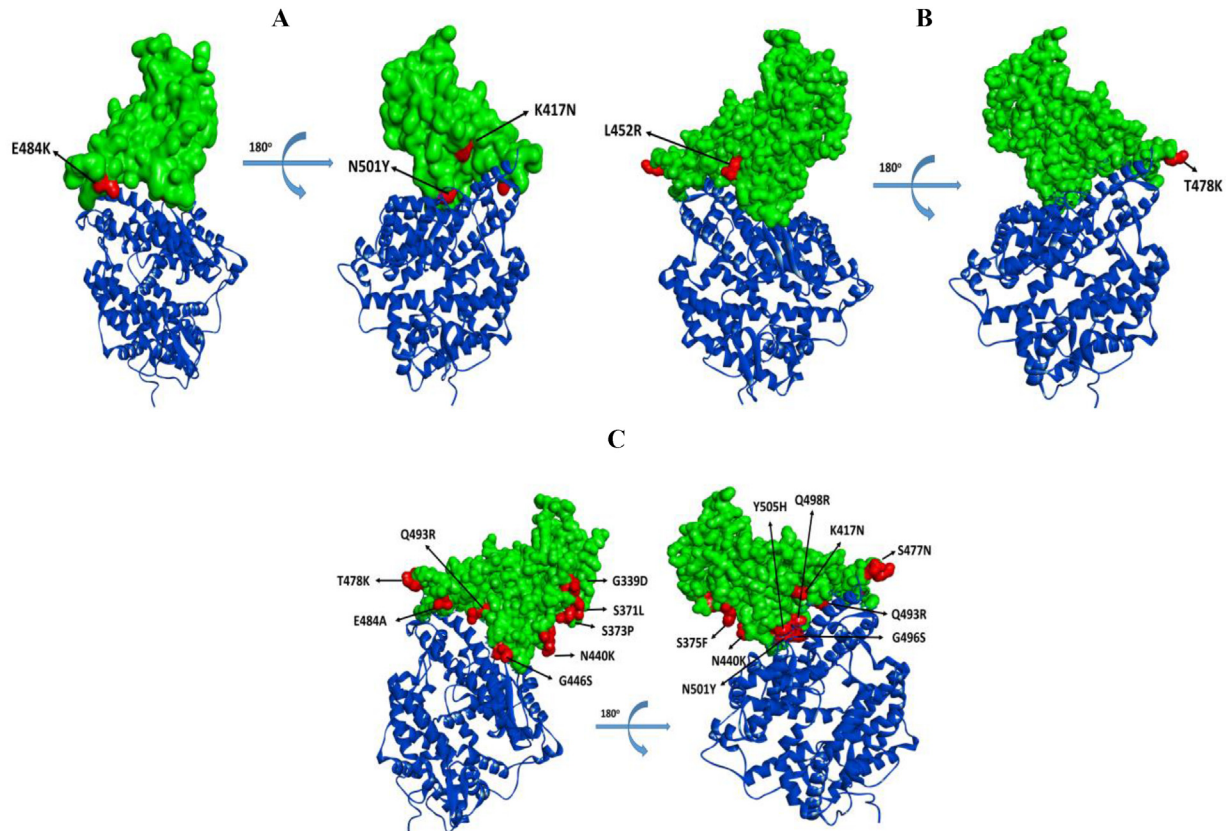


Fig. 2. Mutation mapping of the S-hACE2-SARS-CoV-2 variant complex. (A) Beta-Gamma. (B) Delta. (C) Omicron.

epitopes are important for triggering an immune response, presenting the S protein might be a rationale for several essential vaccines. This is a critical issue concerning the long-term effectiveness of newly developed vaccines. We predicted and compared the B-cell epitopes of the WT and mutant S proteins using a well-established predictive web server tool. The S protein mutants had different epitope variations, which resulted in differences in the efficacy of the antibodies and vaccines (Figs. 2A and 4A). The B-cell epitope profiles of the Gamma, Delta, and Omicron variant mutants were greater than the threshold value (0.5), indicating that these variants are easier to recognize using the BCR of infected cells compared with the WT and Beta variants.

Antigenicity describes the capacity of a virus to bind to antibodies. Variations in the antigenicity at these sites are driven by neutralizing antibodies. Changes in mutation-induced antigenicity play a role in the effectiveness of vaccines [13–15]. We compared the antigenicity fluctuations of the Beta, Gamma, Delta, and Omicron variants. Each variant differed in terms of antigenicity. Omicron antigenicity was severely altered compared with that of

the other variants (threshold value: 1.05) (Figs. 2B and 4B).

Multiple mutations have repeatedly occurred on the SARS-CoV-2 S protein, resulting in the current SARS-CoV-2 variants. To understand the trend in the mutations and the evolution of the structure of the four variants, we compared their protein stability, B-cell epitope, and antigenicity. The first case of Beta was detected in September 2020 in South Africa. Beta was linked with an increased hospitalization rate, immune escape, and death. The Beta variant exhibited no increase in transmissibility [16]. Beta became the most prevalent variant in the second wave of the pandemic after the Alpha variant. Besides D614G, the global mutations in SARS-CoV-2 are known to benefit the virus with faster transmission rates, higher affinity, and higher antigenicity [17]. Beta also gained the E484K and N501Y mutations, which enhanced its binding affinity to hACE2 compared with the WT [18].

The Gamma variant has a lower hospitalization risk than the Beta variant, which is associated with increased transmissibility and immune response escape. The Gamma variant shares some mutations with Beta (N501Y, E484K, and D614G) and has also

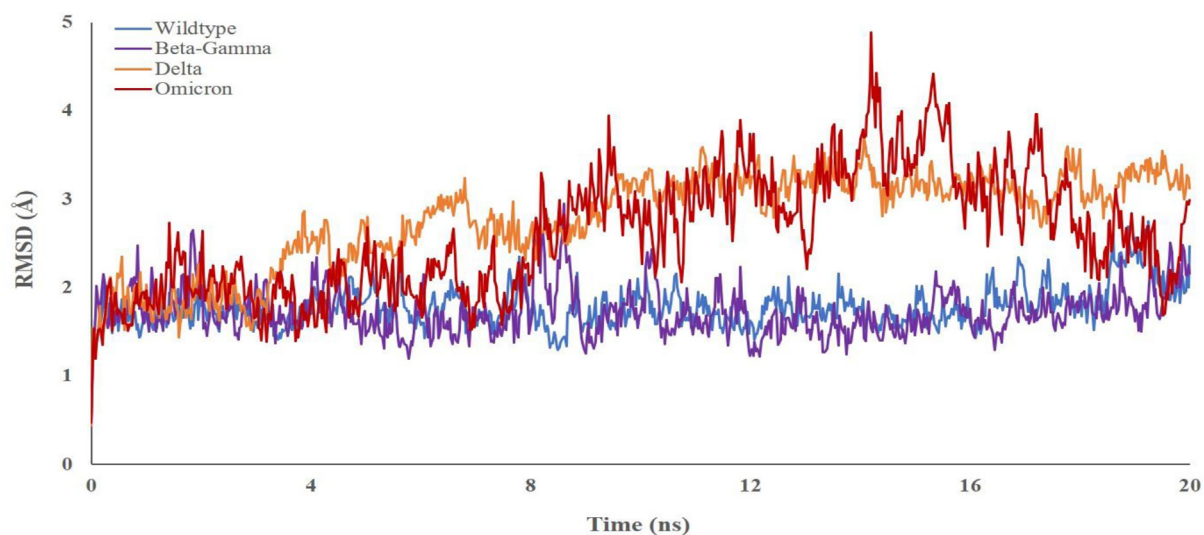


Fig. 3. Backbone fluctuations of the wildtype and mutant S protein–hACE2 complexes. The RMSD value indicates the dynamic of backbone movement of the complex of S protein with hACE2.

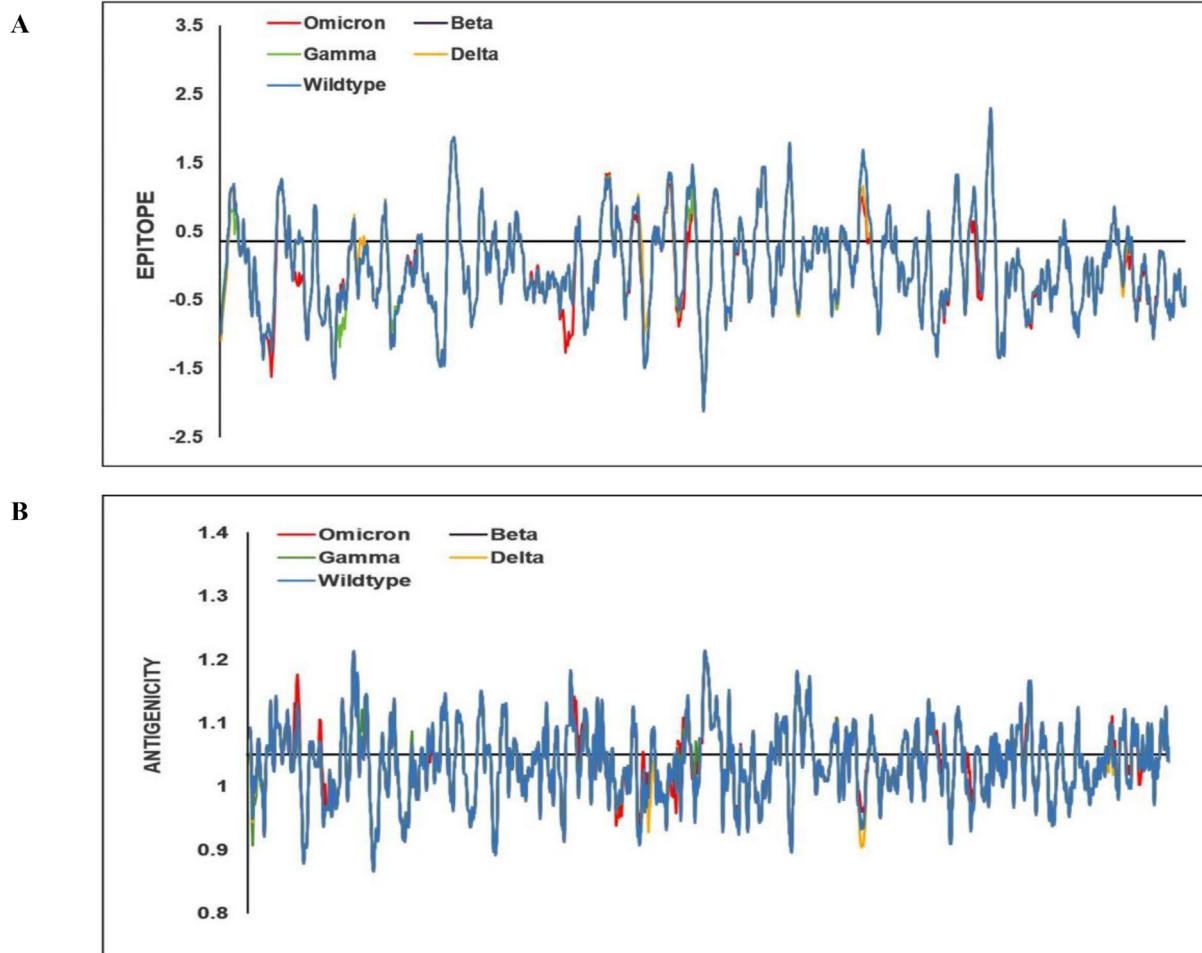


Fig. 4. Linear B-cell epitopes and antigenicity of the SARS-CoV-2 variants. (A) Linear B-cell epitope profile. (B) Antigenicity profile.

gained additional mutations (L18F and K417T). These mutations benefit the virus with evasion of antibody-mediated immunity, reinfection, and increased transmission rates [19].

The Delta variant gained the E484Q and L452R mutations, which are associated with increased hACE2 binding affinity to the RBD. Delta also has a P681R mutation on its S protein sequence, which enhances its immune escape ability. Omicron is the most recent variant circulating in the population and has accumulated the most mutations in its sequence (Fig. 4). Omicron has 30 mutations in the S protein sequence. Omicron has been linked to significantly lower hospitalization and mortality rates than previous variants [20,21].

These results agree with a previous report that suggested that Omicron reduces the neutralization activity of antibodies [22], but its infection fatality rate is lower. Nevertheless, Omicron has a three-fold higher transmissibility rate due to immune escape than previous variants based on South African findings. The mutations appear to alter the dynamics of the protein–protein interactions in the S sequence, which helps stabilize the S protein [18,23–25]. A stable S protein binds more effectively to the ACE2 receptor. A mutation might change the stability of the S protein differently depending on which amino acid undergoes the mutation. Mutations, particularly in the RBD, have various effects on protein stability and ACE2 binding [26]. Different amino acid mutations exert different effects.

Some mutations are associated with higher viral antigenicity [27]. Changes in antigenicity result in the changes in antibody's ability to recognize antigens and reduce the effectiveness of the previous vaccine. This could be the rationale behind the booster vaccine being a prerequisite to protect the population and minimize the effect of the SARS-CoV-2 variants. A vaccine developed for a specific variant may be ineffective or less effective in protecting against future evolving variants. Omicron has been reported to be less protective against infection with two and three doses of vaccination than Delta [28,29]. This phenomenon is mainly caused by the mutations in the S protein, especially on the RBD; Omicron has 30 on its S protein, of which half reside on the RBD [29–31]. This mutation rate is twice that of Delta.

The RMSD results of the S monomer and S protein–hACE2 complex revealed that the Beta and Gamma variants were more likely to be stable than the Delta and Omicron variants, indicating that increasing the number of mutations does not always result in the stabilization of the S protein (Fig. 3). The RMSF results support this finding.

4. Conclusion

Mutations affect the stability of the S protein. However, increasing the number of mutations does not always result in a stable S protein. Moreover, the mutations we induced had different effects on the epitope and antigenicity of each variant. The growing number of mutations in Omicron has significantly changed its antigenicity compared with that of the original Wuhan sequence and other mutant variants, potentially reducing vaccine effectiveness. Our research provides a more comprehensive understanding of the effects of mutation and the evolution of SARS-CoV-2 for long-term vaccine development. Our study should be used as preliminary screening only, as it has computational limitations. Laboratory experimental research is required in future studies.

Conflicts of interest

None of the authors declares any potential conflicts of interest regarding this study.

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