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Informatics in Medicine Unlocked 29 (2022) 100873

Contents lists available at ScienceDirect

Informatics in Medicine Unlocked

journal homepage: www.elsevier.com/locate/imu

SARS-CoV-2 variant surge and vaccine breakthrough infection: A computational analysis

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ARTICLE INFO

Keywords:
COVID-19
SARS-CoV-2
Spike
ACE2
RBD
GRP78

ABSTRACT

Coronavirus Delta variant was first detected in India in October of 2020, and it led to a massive second wave of COVID-19 cases in the country. Since then, the highly infectious Delta strain has been spreading globally. The Delta variant and its sub-lineages showed an increased infection rate with a reduced effect of the potential antibody neutralization. The current work is a modeled computational analysis of the mutated receptor-binding domain (RBD) of the SARS-CoV-2 B.1.617 lineage binding with ACE2 and GRP78 to understand the increased strain transmissibility. The cell-surface Glucose Regulated Protein 78 (GRP78) attached to the mutated ACE2-SARS-CoV-2 Spike RBD complex is modeled. The results showed that GRP78 β-substrate-binding domain weakly binds to the wild-type RBD combined with angiotensin-converting enzyme 2 (ACE2) within the SARS-CoV-2 Spike RBD-ACE2 complex. Both GRP78 and ACE2 bind approximately in the same region on the wild-type SARS-CoV-2 Spike RBD surface. On the other hand, GRP78 strongly binds to the mutated SARS-CoV-2 Spike RBD in the RBD-ACE2 complex through the α-substrate-binding domain instead of β-substrate-binding domain in a different region from that of ACE2. The current findings suggest that blocking the main ACE2 pathway may not prevent the interactions between GRP78 and the mutated SARS-CoV-2 Spike RBD, which might introduce an additional avenue into the virus invasion for the host cell if the ACE2 pathway is blocked by the neutralized antibodies. Hence, the peptide satpdb10668 has been proposed as a potential inhibitor of SARS-CoV-2 attachment and virus invasion into the host cell.

1. Introduction

Viruses are changing constantly due to mutations and new variants are likely to emerge. Occasionally, new variants appear, and they may persist or disappear. Some variants appear to spread more quickly and easily than others. With coronavirus 2019, mutations of the SARS-CoV-2 have been spreading widely over the world since the fall of 2020, several new variants have appeared. The primary variants of concern showed common mutations of the SARS-CoV-2 Spike protein, mostly on the S1 unit, resulting in a higher transmissibility rate compared to the wild type of the SARS-CoV-2. The SARS-CoV-2 variants of the global concern include the Alpha (B.1.1.7) lineage, Beta (B.1.351) lineage, Gamma (P.1) lineage, the Delta lineage, including its three main subtypes (B.1.617.1), (B.1.617.2), and (B.1.617.3), and the latest Omicron (B.1.1.529) variant was recently detected in November 2021 with (BA.1 and BA.2) sublineages [1].

During the time of the vaccine accessibility, questions were raised about the increased number of post-vaccination infections (vaccine breakthrough infection) and the long-term efficacy of the antibodies against the rising transformations within the receptor-binding domain (RBD) of the Spike protein [2–5]. Although SARS-CoV-2 Delta strains first appeared in India, they are currently causing a global sensation. The Delta lineage has mutations in the N-terminal domain (NTD) and the receptor-binding domain (RBD) of the SARS-CoV-2 Spike protein that show resistance to neutralization by some anti-NTD and anti-RBD monoclonal antibodies that may have increased the infectivity or reduced the potential for plasma neutralization, making these lineages spread faster than other variants [6–10]. In the SARS-CoV-2 Spike RBD, mutations emerge on the angiotensin-converting enzyme 2 (ACE2) binding surface's periphery, implying that the virus accumulates mutations there to weaken antibody recognition while retaining ACE2 binding [11–13].

The cell surface Glucose Regulated Protein 78 (GRP78), also known as BiP or HSPA5, is the primary chaperone of the endoplasmic reticulum

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https://doi.org/10.1016/j.imu.2022.100873
Received 9 November 2021; Received in revised form 1 February 2022; Accepted 2 February 2022
Available online 4 February 2022

(ER), which can help with protein folding, protein synthesis, and maturation, and plays an essential role in the regulation of ER stress signaling [14–18]. GRP78 plays a key role as a multiple-function cell surface receptor interacting with many proteins [19–22]. In addition to its role in the cell proliferation, invasion, and metastasis of cancer cells, GRP78 is sensitive to virus recognition through its substrate-binding domain (SBD) and participates in the assembly of its envelope protein [23–26].

The beta substrate-binding domain (β-SBD) of the GRP78 has been determined to be the docking location for the C480–C488 region within the SARS-CoV-2 Spike RBD [27,28]. The GRP78 is predicted to bind to the SARS-CoV-2 Spike nearby the ACE2 host cell receptor [29,30]. The proposed theory is supported by the work of Carlos et al. and Elfiky et al. which found GRP78 binding to ACE2 require the SBD binding domain. They proposed that the substrate-binding domain is significant for attachment [31,32].

The current work aims to calculate the free binding energy of GRP78 and ACE2 with the mutated SARS-CoV-2 Spike RBD Delta lineages (B1.617.1) and (B.1.617.2) to understand the strains' increased transmissibility and to investigate potential antiviral inhibitors. The research proposes that GRP78 establishes an alternative cell entry pathway for the mutated SARS-CoV-2 Spike when the ACE2 pathway is being blocked by the neutralized antibodies. The RBD in the wild-type and the mutant types of the ACE2-SARS-CoV-2 Spike RBD system was simulated and, with the presence of these mutant variants of the viral Spike, the GRP78 binding with the ACE2-SARS-CoV-2 Spike RBD complex (ACE2-RBD) was modeled.

2. Materials and methods

2.1. Molecular dynamics simulation

The SARS-CoV-2 Spike protein mutations (L452R, E484K) found in the SARS-CoV-2 Delta (B1.617.1) lineage and the SARS-CoV-2 Spike protein mutations (K417N, L452R, E484K) found in the SARS-CoV-2 Delta plus (B1.617.2) lineage [33] were prepared using the PyMOL molecular graphics system [34]. The GROMACS-2019 software package [35] and CHARMM36 force field [36] were used to perform the Molecular Dynamic Simulation (MDS) on the wild-type SARS-CoV-2 Spike RBD, as well as the mutated Delta and Delta plus RBD. CHARMM-GUI generated protein topology and parameter files [37–39]. The system was solvated with TIP3P water [40] and the complexes were neutralized by using the Monte-Carlo ion-placing method to add sufficient amounts of K+ and Cl− ions [40]. The system was energy-minimized for 5000 steps with the steepest descent algorithm [41] and equilibrated for 125 ps at a constant number of molecules, volume, and temperature (NVT) before running the simulation. The MDS was then performed for 50 ns at constant temperature (310 K), pressure (1 atm), and the number of molecules (NPT ensemble). The Root Mean Square Deviation (RMSD) of the protein atom backbone and the radius of gyration (Rg) were calculated [42]. The average Root Mean Square Fluctuation (RMSF) was plotted as a function of residue number.

2.2. Docking GRP78 and ACE2-RBD

The ClusPro server is a tool for protein-protein docking [43,44]. The ClusPro 2.0 webserver was used to dock GRP78 (PDB ID: 5E84: A) to the SARS-CoV-2 three types complexes: the wild-type (PDB ID: 6M0J) Spike RBD-ACE2 complex, hereinafter referred to as (WT ACE2-RBD), the Delta mutated RBD-ACE2 complex, hereinafter referred to as (Delta Mut ACE2-RBD), and the Delta plus mutated RBD-ACE2 complex, hereinafter referred to as (Delta plus Mut ACE2-RBD). The binding energy of the complexes was calculated using MM/GBSA of the HawkDock server [45,46]. The PDBePISA server of the Protein Data Bank in Europe was used to analyze the interacting interfaces [47].

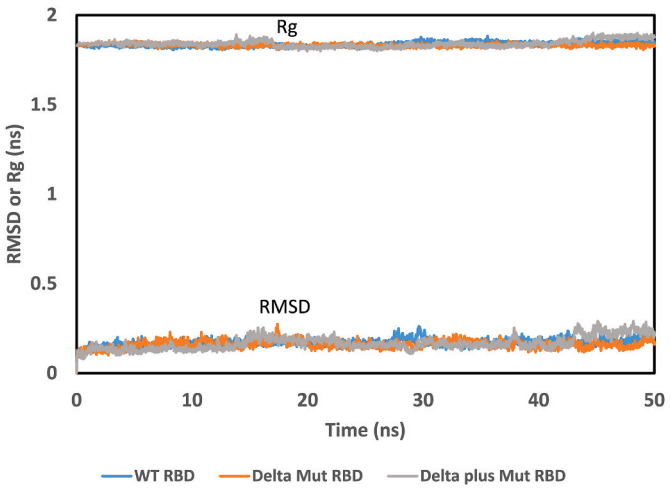

Fig. 1. The Root Mean Square Deviation (RMSD) plots for the backbone atoms and the radius of gyration (Rg) for WT RBD (blue), Delta Mut RBD (red), and Delt plus Mut RBD (grey) over 100 ns simulation as a function of time. The parameters follow a similar pattern. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

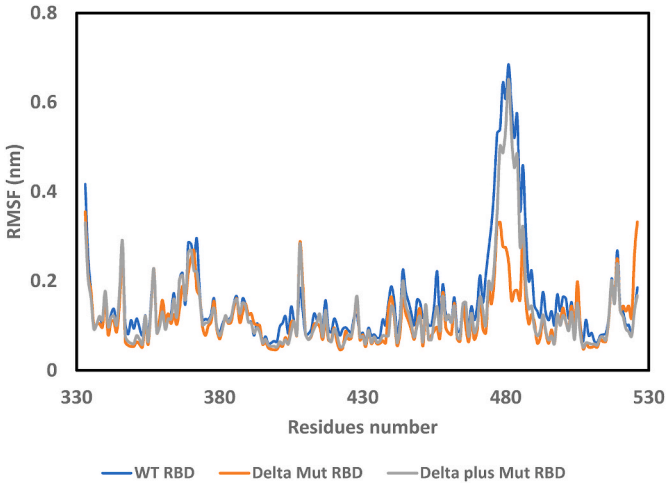

Fig. 2. Plots of the average RMSF per residue over 100 ns of simulation for WT RBD (blue), Delta Mut RBD (red), and Delt plus Mut RBD (grey). The RMSF of Delta Mut RBD and Delta plus Mut RBD residue 408 has increased. There is also a decrease in the RMSF of Delta Mut RBD and Delta plus Mut RBD residues (350–353), (400–404), and (491–497), as well as Delta Mut RBD residues (477–485). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.3. Peptide-protein docking

The Structurally Annotated Therapeutic Peptides Database (SATPdb) was used to find biologically active peptides [48]. The peptides were docked to the Delta mutant RBD and the Delta plus mutated RBD using the ClusPro 2.0 platform. The complexes' binding energy was determined using the HawkDock server's MM/GBSA. The interactions were analyzed using the PDBePISA platform. Molecular dynamics simulation was used to assess the stability of the produced complexes.

3. Results and discussion

In the current work, the ACE2-SARS-CoV-2 Spike RBD complex was docked using the RBD in the wild-type and the mutated Delta lineages.

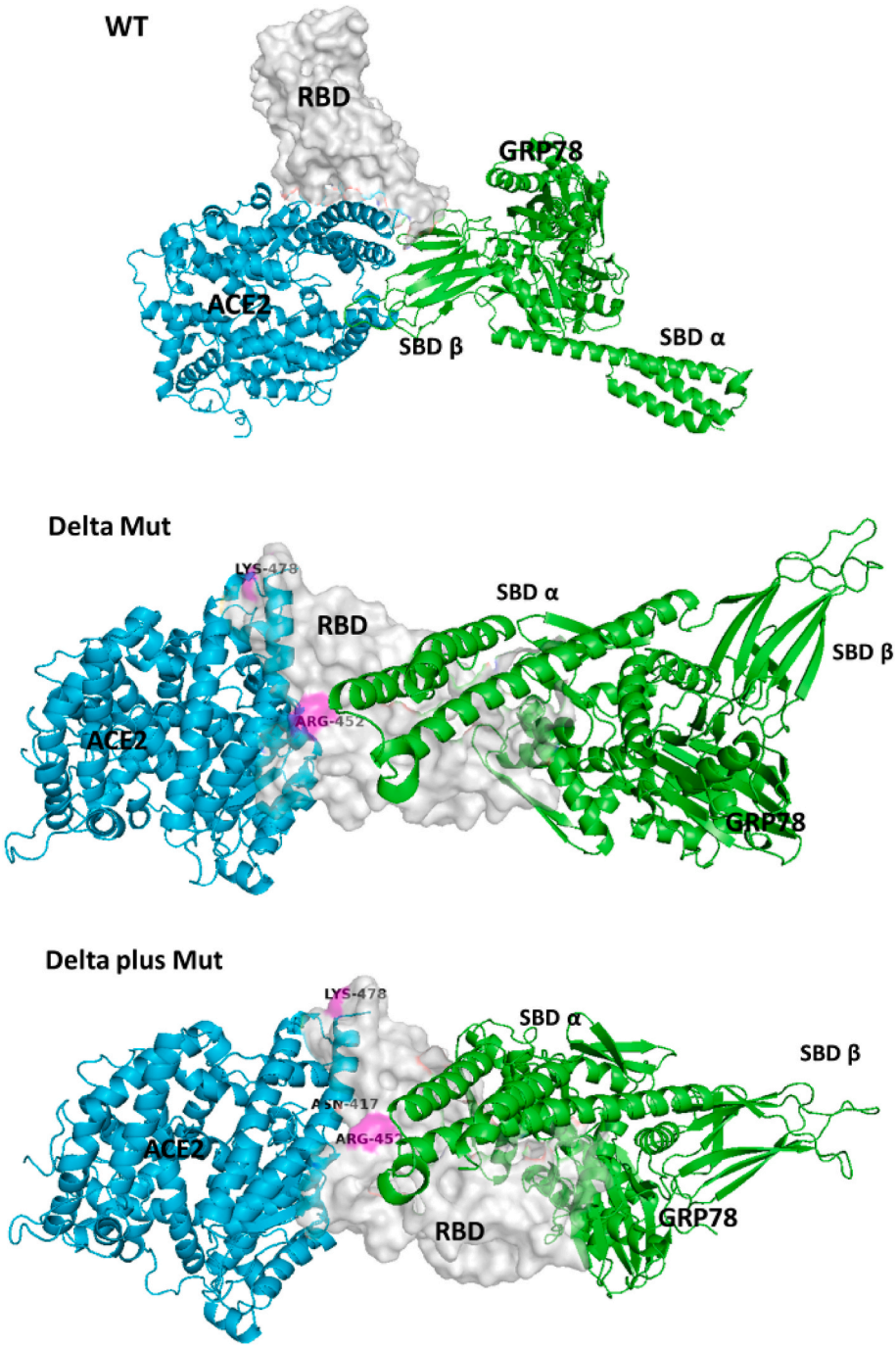

Fig. 3. Docking of GRP78 to WT RBD-ACE2, Delta Mut RBD-ACE2, and Delta plus Mut RBD-ACE2 using the ClusPro 2.0. GRP78 SBD β weakly binds to WT RBD and ACE2 while GRP78 SBD α strongly binds to Delta Mut RBD and Delta plus Mut RBD region III.

In addition, the mutant variants of the viral Spike and the cell-surface GRP78 were docked with the ACE2-SARS-CoV-2 Spike RBD complex. Molecular Dynamics Simulations on the SARS-CoV-2 Spikes of the WT RBD, Delta Mut ACE2-RBD, and Delta plus Mut ACE2-RBD were performed. A plot of the RMSD values of the WT RBD (blue), Delta Mut RBD (red), and Delta plus Mut RBD (grey) are displayed in Fig. 1. The calculated Rg values over the simulation time scale are displayed in Fig. 2. The results show that the parameters roughly have the same pattern for the WT RBD (blue), Delta Mut RBD (red), and Delta plus Mut RBD (grey) over the simulation time. The average RMSF plotted over 50 ns per residue for RBD (blue), Delta Mut RBD (red), and Delta plus Mut RBD (grey) are displayed in Fig. 3. The results show that there is an increase in RMSF of residue number 408 of the Delta Mut RBD and Delta plus Mut RBD. The RMSF of residues between (350–353), (400–404), and (491–497) of the Delta Mut RBD and Delta plus Mut RBD, as well as residues between (477–485) of the Delta Mut RBD, noticeably decreases.

GRP78 was docked to the WT ACE2-RBD, Delta Mut ACE2-RBD, and Delta plus Mut ACE2-RBD using the ClusPro 2.0. The resulting complexes were ranked by the binding energies calculated using MM/GBSA, hence the complexes that have the highest binding energies were selected. Fig. 3 illustrates the complexes formed. The binding energies between GRP78 and ACE2 with RBD within the three complexes were calculated using MM/GBSA. The interacting interfaces were analyzed using the PDBePISA server by predicting the hydrogen bonds and salt bridges and Table 1 lists the interactions formed.

The binding energies of ACE2 with the Delta Mut RBD (−103.19

Table 1
The binding energy of GRP78 and ACE2 with RBD was calculated using MM/GBSA, and the interactions are analyzed by using PDBePISA.

WT ACE2-RBD-GRP78 complex							
ACE2-RBD interactions (−87.5 ± 0.85) kcal/mol				GRP78-RBD interactions (-18.5 ± 0.19) kcal/mol			
14 Hydrogen bonds		4 Salt bridges		3 Hydrogen bonds		No Salt bridges	
ACE2 residues	RBD residues	ACE2 residues	RBD residues	GRP78 residues	RBD residues	GRP78 residues	RBD residues
GLN24 ASP30 GLU35 GLU37 ASP38 GLN42 TYR83 LYS353	**LYS417 GLY446 TYR449 ASN487 TYR489 GLN493 GLY496 GLN498 GLY502 TYR505**	**ASP30 LYS 31**	**LYS417 GLU484**	**LYS435**	**PRO479 YS480 ASN 481**	-	-
Delta Mut ACE2-RBD-GRP78 complex							
ACE2-RBD interactions (-103.19 ± 0.07) kcal/mol				**GRP78-RBD interactions (-148.29 ± 0.42) kcal/mol**			
18 Hydrogen bonds		**4 Salt bridges**		**20 Hydrogen bonds**		**21 Salt bridges**	
ACE2 residues	**RBD residues**	**ACE2 residues**	**RBD residues**	**GRP78 residues**	**RBD residues**	**GRP78 residues**	**RBD residues**
GLN24 ASP30 HIS34 GLU35 GLU37 ASP38 TYR41 GLN42 TYR83 LYS353	**LYS417 GLY446 TYR449 ASN487 TYR489 GLN493 GLY496 GLN498 THR500 ASN501 GLY502 TYR505**	**ASP30**	**LYS 417**	**GLU73 ASN82 ARG283 GLU310 ARG554 ARG558 ASN559 GLU562 GLU 599 GLU602 SER603**	**ARG 355 GLY381 LYS386 ARG 357 LYS462 ARG 466 GLU516 LEU518 ALA 520**	**ARG290 GLU310 ARG554 ARG558 GLU562 GLU599 GLU602 ASP606**	**ARG346 RG357 LYS386 LYS 462 ARG466 GLU516**
Delta plus Mut ACE2-RBD-GRP78 complex							
ACE2-RBD interactions (-93.06 ± 0.57) kcal/mol				**GRP78-RBD interactions (-113.73 ± 0.66) kcal/mol**			
16 Hydrogen bonds		**2 Salt bridges**		**21 Hydrogen bonds**		**25 Salt bridges**	
ACE2 residues	**RBD residues**	**ACE2 residues**	**RBD residues**	**GRP78 residues**	**RBD residues**	**GRP78 residues**	**RBD residues**
GLN24 ASP30 HIS34 GLU35 GLU37 ASP38 TYR41 GLN42 TYR83 LYS353	**LYS417 GLY446 TYR449 ASN487 TYR489 GLN493 GLY496 GLN498 THR500 GLY502 TYR505**	-	-	**ASP56 GLN57 ASN82 LYS152 GLU155 YR160 LYS552 ASP556 ASN559 GLU599 GLU560 GLU562 SER563 TYR566 ASP574 GLU576 GLU602**	**ARG346 ARG355 ARG357 ASP389 THR393 ASN394 ASP428 ARG466 SER469 THR470 LEU517 LEU518 HIS519**	**LYS46 ARG49 GLU51 GLU155 LYS552 ASP556 GLU560 LU562 ASP574 GLU576**	**ARG346 ARG357 LYS386 ASP389 ARG466 GLU516 HIS519**

kcal/mol) and the Delta plus Mut RBD (−93.06 kcal/mol) were found to be higher than that of the WT RBD (−87.5 kcal/mol). The ACE2 formed 14 hydrogen bonds and 4 salt bridges with the WT RBD. On the other hand, ACE2 formed 18 hydrogen bonds and 4 salt bridges with the Delta Mut RBD, while ACE2 formed 16 hydrogen bonds and two salt bridges with the Delta plus Mut RBD. The number of the interacting interfaces might have explained here the increased transmissibility of the mutated SARS-CoV-2 Delta and Delta plus lineages.

The results of the GRP78 binding to RBD are found to be consistent with the study findings of Ibrahim et al. [49], which explained that GRP78 binds to the SARS-CoV-2 Spike in 4 regions, among which regions III (C391–C525) and IV (C480–C488) show stronger Spike RBD affinity. The results show that the β-substrate-binding domain of GRP78 weakly binds to region IV of the WT RBD (C480–C488) with binding energy (−18.5 kcal/mol) forming three hydrogen bonds with PRO479, CYS480, and LYS481. The results are listed in Table 1. The GRP78 and ACE2 roughly bind to the WT RBD surface at the same sites and bind to each other. The bindings are illustrated in Fig. 3. The same proposition has been confirmed by Aguiar et al. [50] who explained that the GRP78 binding site overlaps with the ACE2 binding site, even though the residues involved in the interactions might have been a little different. Consequently, GRP78 stabilizes the binding of the RBD and ACE2, which enhances the effective entry of the virus, but cannot be considered as the self-receptor of the WT SARS-CoV-2. On the other hand, GRP78 strongly

Table 2
The three peptides selected from the Structurally Annotated Therapeutic Peptides Database inhibit the interactions formed between GRP78 and both SARS-CoV-2 Delta Mut RBD and SARS-CoV-2 Delta plus Mut RBD.

Peptides S-ID	Sequence	Function	% Helix	% Sequence similarity with GRP78 α12 Helix
satpdb10668	WQEWERKVDFLEENITALLEEAQIQQEKNMYELQK	Antiviral, antimicrobial	94.3	16.0
satpdb10029	TLGEWYNQTKDLQQKFYEIIMDIEQNNVQGKKG	Antiviral, antimicrobial	93.9	18.18
satpdb10328	LSELDDRADALQAGASQFETSAAKLKRKYWWKN	Antiviral, antimicrobial	93.9	16.67

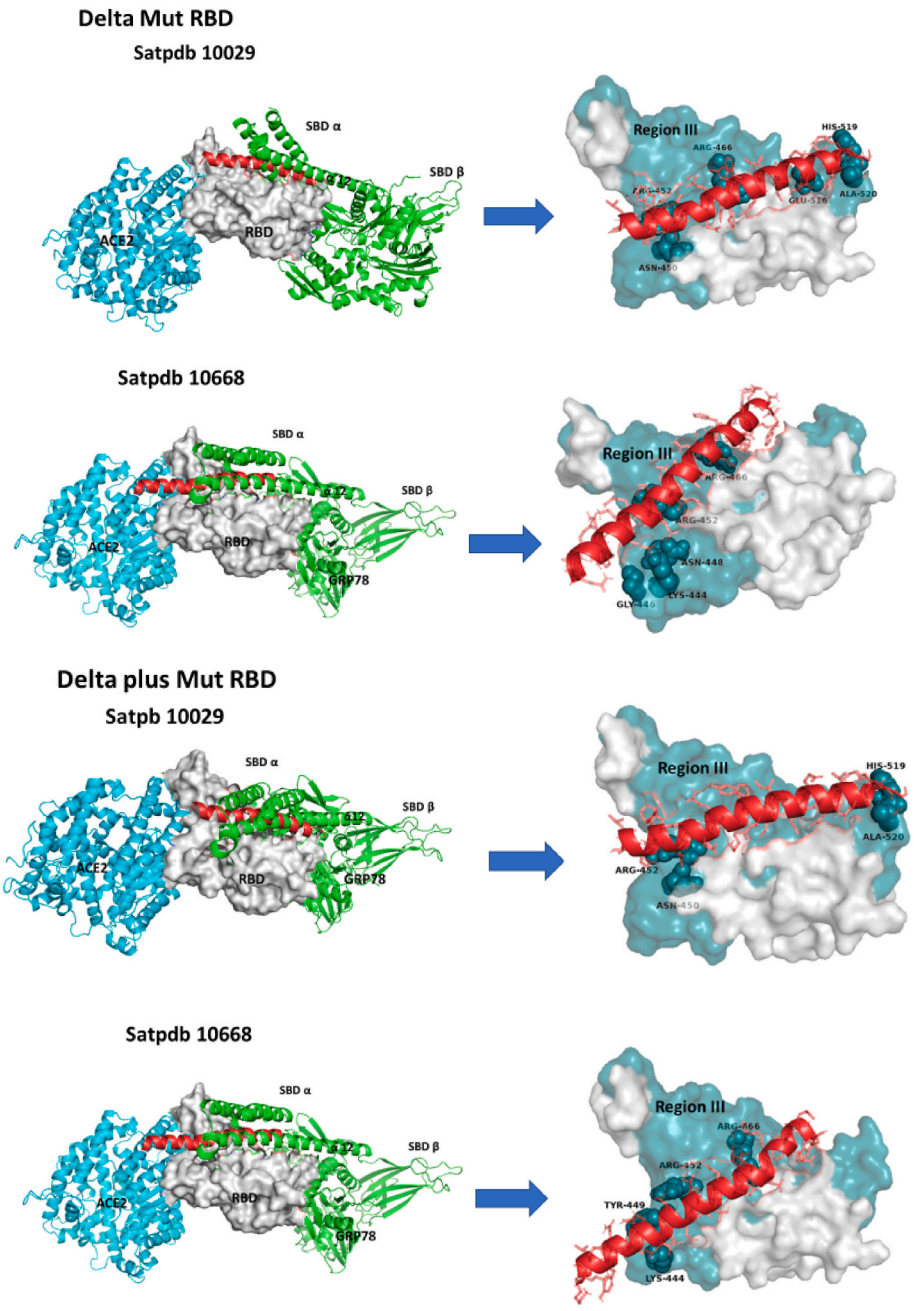

Fig. 4. Peptides satpdb10029 and satpdb10668 docked to Delta Mut RBD and Delta plus Mut RBD using the ClusPro 2.0. The two peptides docked at the same position of GRP78 SBD α forming hydrogen bonds and salt bridges with RBD region III residues.

binds to the Delta Mut RBD (−148.29 kcal/mol) and the Delta plus Mut RBD (−113.73 kcal/mol) in region III (C391–C525) through the α-substrate-binding domain instead of β-substrate-binding domain. GRP78 formed 20 hydrogen bonds and 21 salt bridges with the Delta Mut RBD and formed 20 hydrogen bonds and 24 salt bridges with the Delta plus Mut RBD. Most of the interactions between the α-SBD, especially α12 helix, of GRP78 and the residues in RBD region III. The interacting interfaces are listed in Table 2. GRP78 binds to the Delta and Delta plus mutant of the RBD at a completely different site from ACE2 but does not bind to ACE2 as displayed in Fig. 3. This means that the waning

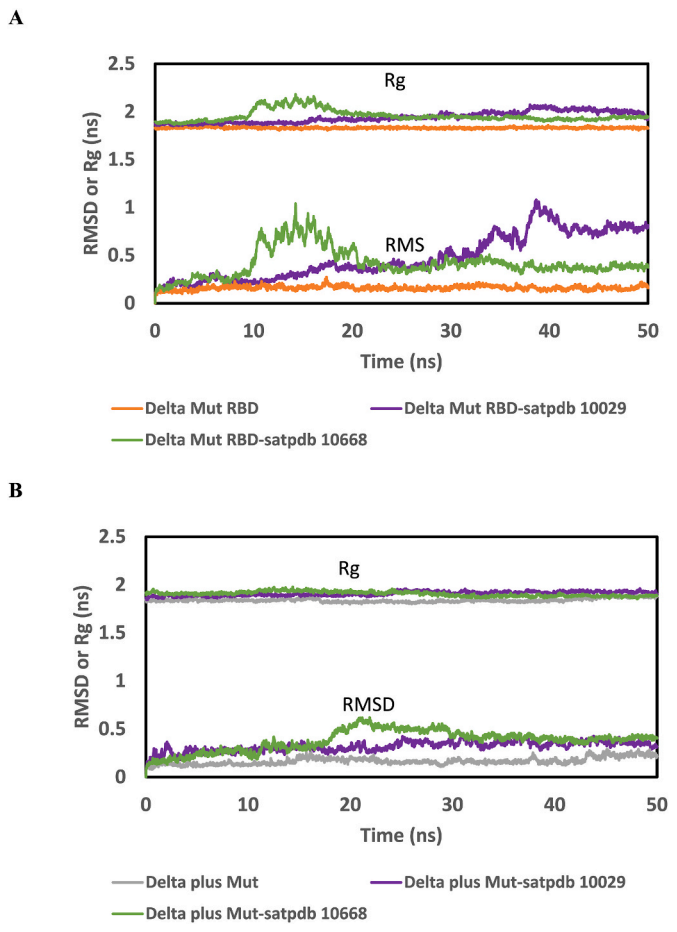

Fig. 5. (A) Plots of Root Mean Square Deviation (RMSD) for the backbone atoms and the radius of gyration (Rg) for Delta Mut RBD (red), Delta Mut RBD-satpdb10029 (violet), and Delta Mut RBD-satpdb10668 (green) over 100 ns simulation as a function of time. The Delta Mut RB-satpdb10029 complex did not stabilize over simulation time, whereas the Delta Mut RB-satpdb10668 complex was unstable between 10 and 20 nm before stabilizing time. **(B)** The same Plots for Delta plus Mut RBD (grey), Delta plus Mut RBD-satpdb10029 (violet), and Delta plus Mut RBD-satpdb10668 (green) over 100 ns simulation as a function of time. Delta plus Mut RB-satpdb10668 is a more stable complex than Delta plus Mut RB-satpdb10029. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

immunity or the neutralized antibodies are blocking the main ACE2 pathway, which in turn would not prevent the interaction between GRP78 and the Delta Mut RBD and Delta plus Mut RBD, which, consequently, would have established an alternative route for the virus entry. The binding analysis explains the potential reduction in the neutralization of those SARS-CoV-2 B.1.1.7 Lineages after receiving monoclonal antibody treatments or the neutralized antibodies which target the binding of RBD with ACE2. That implies that the treatment for the new variants of the SARS-CoV-2 virus requires additional blockers of the α-substrate-binding domain of GRP78 and RBD region III (C391–C525).

SARS-CoV-2 has changed through time, with mutations that make a significant difference soon becoming dominant lineages. Neutralization of mutated viruses is reduced compared with the wild-type strains [51,52]. Recent studies focused on protection against SARS-CoV-2 variants of concern using alternative epitopes [53,54] which may boost the immunity against the new variants. The findings of this work are rather suggesting the use of therapeutic peptides to block the interaction between the RBD of the virus and the GRP78. In an attempt to search for peptide inhibitors, we screened available databases for peptides that block region III of RBD mutated Delta and Delta plus lineages in the Structurally Annotated Therapeutic Peptide Database (SATPdb), accessible at (http://crdd.osdd.net/raghava/satpdb/). All antiviral peptides with a helix structure greater than 90% were carefully chosen and their sequences were aligned with the α12 helix of GRP78. The three peptides with the highest similarity percentage were selected. A list of the peptides and their sequences is shown in Table 2. The peptides were then docked against the mutated SARS-CoV-2 Spike RBD of the Delta and Delta plus lineages using ClusPro 2.0. The generated complexes were ranked by the binding energies computed using MM/GBSA, and the complexes with the highest binding energies were selected. Peptides satpdb10668 and satpdb10029 were chosen to target the Delta Mut RBD Region III as shown in Fig. 4. Peptide satpdb10029 formed 8 hydrogen bonds and 4 salt bridges with the Delta Mute RBD region III residues (ASN450, ARG452, ARG466, GLU516, HIS519, and ALA520), at the same time. Peptide satpdb10029 also formed 6 hydrogen bonds and 4 salt bridges with the Delta plus Mute RBD region III residues (ASN450, ARG452, HIS519, and ALA520). Peptide satpdb10668 formed 7 hydrogen bonds and 8 salt bridges with the Delta Mut RBD region III residues (LYS444, ASN448, ARG452, and ARG466), whereas it formed 8 hydrogen bonds and 8 salt bridges with the same residues of the Delta plus Mut RBD region III. These peptides could be used as blocking agents for the GRP78 receptor binding to the SARS-CoV-2 Spike RBD of the mutated Delta and Delta plus lineages.

The molecular docking procedure creates instantaneous interactions between peptides and proteins, which might be unstable [55]. Molecular dynamic simulations can provide information about the stability of the formed complexes' molecular interactions. The Delta Mut RBD and Delta plus Mut RBD in complex with peptides satpdb10029 and satpdb10668 were used to perform the molecular dynamics simulations. Using the RMSD approach, the stability of the complexes was evaluated for the backbone atoms concerning the original starting structures [56]. Additionally, the stability of those complexes was assessed further using the Rg plot [56]. A plot of the RMSD and Rg values over the simulation time scale is displayed in Fig. 5. The Delta Mut RBD-satpdb10029 complex did not stabilize over the simulation time, whereas the Delta Mut RBD-satpdb10668 complex was unstable between 10 and 20 nm before becoming stable. The Delta plus Mut RBD-satpdb10029 and Delta plus Mut RBD-satpdb10668 complexes were roughly stable over the simulation time. The findings suggest peptide satpdb10668 as a potential inhibitor of the mutated SARS-CoV-2 Delta and Delta plus lineages.

4. Conclusion

The coronavirus disease of 2019 (COVID-19) remains a worldwide health concern due to its high infection rate. Genetic mutations of the SARS-CoV-2 Spike protein keep emerging with time and the variants keep spreading rapidly. The Delta variants and their sub-lineages are reported to have increased infection transmissibility and build waning immunity or resistance. In this study, we analyzed the binding energy of the mutated SARS-CoV-2 Spike protein of the Delta Mut RBD and Delta plus Mut RBD with GRP78, which formed an alternative pathway for the cell entry when the ACE2 pathway is blocked. The findings of this study imply that, in addition to the neutralized antibodies, inhibitors are further needed to block the genetic lineages occurring. SARS-CoV-2 virus adhesion and invasion into the host cell are suggested to be inhibited by the peptide satpdb10668.

Consent statement/Ethical approval

Not required.

Funding

No funding was provided.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors wish to acknowledge the Department of Biophysics, Faculty of Science, Cairo University for facilitating the work.

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