Structural Analysis And Molecular Characteristics of SARSCoV-2 Spike Protein Following S494P Point Mutation: Molecular Dynamics Study

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Abstract

The emergence of some mutations in the SARS-CoV-2 receptor binding domain (RBD) can increase the spread and pathogenicity due to the conformational changes and increase the stability of Spike protein. Due to the formation of different strains of SARS-CoV-2 by mutations, and their catastrophic effect on public health, the study of the effect of mutations by scientists and researchers around the world is inevitable. According to available evidence, the S494P variant is observed in several SARS-CoV-2 strains from Michigan, USA. To investigate how the S494P natural mutation alters receptor binding affinity in RBD, we performed structural analysis of wild-type and mutant spike proteins using some bioinformatics and computational tools. The results show that S494P mutation increases the spike protein stability. Also, applying docking by HADDOCK displayed higher binding affinity to hACE2 for mutant spike than wild type possibly due to the increased β -strand and Turn secondary structures which increases surface accessible surface area (SASA) and chance of interaction. The analysis of S494P as a critical RBD mutation may provide the continuing surveillance of spike mutations to aid in the development of COVID-19 drugs and vaccines.

Introduction

SARS-CoV-2 belongs to the subfamily *Coronavirinae*, a member of the family *Coronaviridae*, is enveloped, positive-strand RNA viruses and can infect both animals and humans [1]. The viruses are subdivided, based on genotypic and serological characters, into four genera: *Alpha*-, *Beta*-, *Gamma*-, and *Deltacoronavirus* [2, 3]. Thus far, all identified CoVs that can infect humans belong to the *alphacoronaviruses* (alphaCoVs) hCoV-NL63 and hCoV-229E and the *betacoronaviruses* (betaCoVs) HCoV-OC43, HKU1, SARS-CoV, and MERS-CoV [4]. In human, SARS-CoV-2 like SARS and MERS causes respiratory disease called coronavirus disease 2019 (COVID-19) [5]. The first known genus was identified in Wuhan, China, in December 2019 [6, 7]. Since then, many variants have formed and spread around the world, leading to the global pandemic attributed to COVID-19, which is known as one of the deadliest pandemics in history. In addition, another consequence of the COVID-19 is the severe recession in the global economy resulting in a dramatic loss of livelihoods and income on a global scale. Under these circumstances, the study and research of scientists and researchers around the world has led to a great deal of knowledge about SARS-CoV-2. This knowledge will greatly help to find and improve vaccines and therapeutics to halt the coronavirus pandemic.

Among several viral proteins, one such major vaccine and antibody target is the spike glycoprotein (S-protein) of the coronavirus which has an essential role in virus entry into the host cell and pathogenesis of SARS-CoV-2 [8, 9]. The length of the spike protein is about 20 nm and it is a homotrimer protein with two functional subunits S1 and S2 per monomer. The S1 subunit forms the spike head and recognizes human angiotensin-converting enzyme 2 (hACE2) [10]. The receptor-binding domain (RBD) which recognizes the host cell surface receptor (hACE2) and is responsible for virus attachment at the cell surface, is located within S1 [11, 12]. The S2 subunit forms a stem that anchors the spike in the viral envelope and fuses the viral particles to the cell membrane. RBD has a region called the receptor binding motif (RBM). RBM directly binds to the viral binding motif (VBM) in hACE2 [13]. The important structural features of the spike are illustrated in Fig. 1. Spike variants that have a greater virus-receptor interaction are easily spread and become infections [12]. Given what may be an exponential increase in infections, there is expected to be a higher rate of morbidity or mortality. According to available evidence, the S494P variant is observed in several SARS-CoV-2 strains from Michigan, USA [14]. However, no detailed study has been done on structural changes of spike protein due to S494P mutation and binding affinity for hACE2. Here, for a better understanding, we investigated the structural and molecular properties of S494P mutation using of bioinformatics tools and molecular dynamics simulation.

Methods

2.1. Stability investigation

There is a close relationship between stability and structural energy of proteins. It is also determine the stability changes caused by the amino acid substitution of proteins [15]. Hence, one of the most common approaches to trace the effect of mutations on protein stability is structural energy calculation. To date, numerous computational tools have been developed to predict the effect of mutations on stability. However, only a few of these tools have been employed to improve protein stability experimentally through the use of point mutation [16]. So, we selected two popular tools means FoldX (version 5) plugin for YASARA [17] and PoPMuSiCv3.1 [18] to calculate and compare the energy of the structure in the wild and mutant spike. FoldX calculate the energy of the structure by applying linear combination of physics and statistics-based energy terms algorithms [19]. It provides useful information about the characteristics of protein conformations by calculating the different terms of energy.

2.2. Molecular Dynamics evaluation

Molecular dynamics simulation is a high speed, and flexibility of approach carried out to find out the behavior and movement of atoms and molecules over a given period of time [20]. The 3D structure of spike with PDB ID: 6M0J was retrieved from the protein databank (<http://www.rcsb.org/pdb>) [21]. Simulations for wild-type (WT) and mutant spike proteins were performed using the GROMACS package (version 2019.1) with Amber03 force field [22–24] and run for 100 ns. The 6M0J X-ray structure was placed in a cubic box containing approximately

7285 SPC 216 water molecules and using periodic boundary condition. The distance from the cubic-box edges was set to 1 nm. In order to neutralize the net charge of systems 2Cl⁻ ions were added. Periodic boundary conditions (PBC) were exerted in all three directions of the system. Then, the entire system was minimized using the steepest descent of 1000 steps followed by the conjugate gradients of 10000 steps [25]. In the equilibration step an NVT ensemble with a constant temperature of 300 K for 200 ps, and NPT ensemble with a constant pressure of 1 bar for 500 ps were applied. Berendsen [26] and Parrinello-Rahman algorithms [27] were applied to keep the pressure and temperature constant at 1 bar and 300 Position restraint process was used in NVT and NPT ensemble steps. We performed two independent MD runs, from wild and mutant structures in order to ensure that results are significant for the relevant mutation. We then computed the comparative analysis of structural deviations in wild and mutant structures. Root-mean-square-deviation (RMSD), Root-mean-square-fluctuation (RMSF), and surface accessibility surface area (SASA) were measured for target structures..

2.3. Principal component analysis (PCA) and Free energy landscape (FEL)

It is used by principle component analysis (PCA) as a standard tool in statistical mechanics to determine the correlated motions of the residues to a set of linearly uncorrelated variables called principle component. This method was carried out to construction the linear transformation of characteristics of the covariance matrix and reduction of the instantaneous linear correlations among the coordinates of the simulated proteins. In this study, Cα atoms of target proteins were selected to estimation of fluctuations, provides significant characteristics to essential internal motions. Results obtained from PCA were subjected to free energy landscape assessment (FEL), to describe the probability energy distribution of one or more collective variable of the protein system, which helps to visualize the stability of different conformations for a protein [28]. Extraction of 2D representations of the FEL from the trajectory was carried out using g_sham in GROMACS [29].

2.4. Immunogenicity characteristics of spike Protein

Immunogenicity is a humeral or cellular immune response in the body of a human administered a drug recognizes it as foreign. Cytotoxic CD8 + T lymphocytes (CTLs) directly kill virus-infected cells in the respiratory tract. Identification of epitopes of viral antigens that can be effectively delivered to T lymphocytes to elicit a protective response for the host is important in vaccine development [30]. At the same time, antigen-supplying cells (APCs) play a major role in the presentation of antigenic peptides by human leukocyte (HLA) class I molecule to CD8 + lymphocytes [31]. HLA molecules with very high polymorphisms can present viral antigen peptides to T lymphocytes, causing a variety of immune responses in different individuals [32]. Due to structural changes of point mutation proteins, it is expected that the immunogenicity of the mutant spike proteins could also be changed. In this study, the IEDB [33] server was used to evaluate and compare the immunogenicity of the wild type and mutant spikes. T-cell epitope prediction was accomplished by MHC I binding prediction options.

2.5. Docking

To investigate the effect of the S494P mutation on the ability of spike to bind to the hACE2 receptor, docking process was utilized. HADDOCK is a flexible docking program where experimental or bioinformatics information is directly used to predicting the binding affinity of protein-protein interactions [34]. It is user-friendly software, which uses biochemical or biophysical information to enhance sampling and scoring [34]. We have implemented some restrictions to run this software, the active and passive residues were our restrictions. According to [35], and analysis of some existing crystal structures with PDB ID 6VW1, 6LZG, and 6M17 active residues were identified (Table 1). During docking, the solvated docking mode was activated and active residues were considered as the fully-flexible segment. Passive residues that were close to the active residues were chosen automatically. The cluster with the best score was used during the docking for analysis. The LigPlot v.2.2 was applied to determine schematic diagrams of the protein-protein interactions [36].

Table 1
Name and position of the SARS-CoV-2 and hACE2 contact residues.

SARS- CoV-2	K417, G446, Y449, Y453, L455, F456, A475, F486, N487, Y489, Q493, G496, Q498, T500, N501, G502, Y505
hACE2	Ser19, Q24, F28, D30, K31, H34, E35, E37, D38, Y41, Q42, L79, M82, Y83, N330, K353, G354, D355, R357, R393

Results And Discussion

3.1. Stability investigation

The most important forces for protein stability are intramolecular interactions, i.e. disulfide bridges, ion interactions, hydrogen bonds, hydrophobic interactions and core packing [37]. The core function of FoldX and PoPMuSiC, the empirical force field algorithm, is based on free energy (ΔG) terms aiming to calculate the change of ΔG in kcalmol⁻¹ (Eq. (1)).

$$\Delta G = a \Delta G_{vdw} + b \Delta G_{solvH} + c \Delta G_{solvP} + d \Delta G_{wb} + e \Delta G_{hbond} + f \Delta G_{el} + g \Delta G_{kon} + h T \Delta S_{mc} + k T \Delta S_{mc} + l \Delta G_{clash}.$$

(1)

In this study, both PoPMuSiC and FoldX outputs showed that the P494 increases the stability of spike protein conformation. The $\Delta\Delta G$ calculated by PoPMuSiC for S494P mutation is $-0.02 \text{ kcalmol}^{-1}$. ($\Delta\Delta G < 0$ means stabilizing mutation). The total energy calculated by FoldX was 60.62 and $56.41 \text{ kcalmol}^{-1}$ for wild type, and S494P spike protein, respectively. According to Table 2, substitution of S494 by P decreases SideHbond from -41.40 to $-50.56 \text{ kcalmol}^{-1}$. The lower energy content of S494P mutation indicates that P367 mutation increases the stability of spike protein. So, it can impact the performance of the SARS-CoV-2.

Table 2 Energy parameters measured by FoldX for the native and mutant spike. (the unit of all energy terms is kcalmol^{-1}).

FoldX	BackHbond	SideHbond	Energy_VdW	Electrostatic	Energy_SolvP	Energy_SolvH	Energy_vdwc_lash	Energy_torsion	Entropy_sidec	Entropy_mainc
S494	-101.05	-41.40	-220.30	-14.66	309.78	-278.13	16.16	6.42	112.23	301.00
P494	-101.04	-50.56	-220.55	-14.68	308.43	-279.61	16.08	6.45	112.16	300.72

3.2. Molecular dynamics simulation analysis

We next performed 100 ns MD simulation to analyze carefully the structural consequences of mutating residue S494P, which is naturally occurred in spike protein.

The stability of the protein relative to its conformation can be determined by the deviations produced during the simulation trajectory. The RMSD value is used for measuring the difference between the backbones of a protein from its initial structural conformation to its final position. The smaller the deviations, the more stable the protein structure. From the RMSD simulation shown in Fig. 2.A, it can be observed that the systems equilibrated after 40 ns. Also, the structure of the mutant shows a significant deviation from wild type. The average value of fluctuations in backbone RMSD during the last 60 ns is depicted in Table 3. The lower RMSD values for mutant structure indicate that the overall topology has changed so that S494P shows a stable structure than wild type system.

The residue-based root mean square fluctuation (RMSF) of the backbone for the S494P displayed whit less flexibility in the residue number 415–485, which is a receptor binding motif (RBM) region of spike protein compared to the wild type. This indicates that the secondary structure of RBD was stable. Accordingly, the RMSF results also confirm that S494P system is more stable than wild type (Fig. 2.B).

Table 3 The average value of RMSD, and RMSF during the simulation for wild type and mutant structures.

Parameters	Wild type	S494P
RMSD ¹	$0.494 \pm 0.078 \text{ (nm)}$	$0.410 \pm 0.049 \text{ (nm)}$
RMSF ²	$0.163 \pm 0.12 \text{ (nm)}$	$0.147 \pm 0.14 \text{ (nm)}$
SASA	108.40 ± 2.03	$110.9 \pm 2.47 \text{ (nm)}$
¹ The average value during the last 60 ns		
² The average value is amino acids between 340–515.		

Another important structural parameter, that provides important information about the structural plasticity of proteins, is the time-dependent secondary structures which calculated using DSSP classification of secondary structure elements. Analysis of DSSP results shows that in the mutant structure the tendency to forming of β -sheet, bend and turn secondary structures have increased but coil and α -helix reduced (Fig. 3). In general, it can be said that the S494P mutation cause an increase the sum of the regular structures. Since the tow bend and turn secondary structures tend to be at the protein surface, it can increase the available surface area of the mutant spike and the chance of the interaction of mutant spike with wild type receptor protein. Determining the amount of SASA also confirm this claim (Fig. 3).

3.3. Free energy landscape (FEL)

To indicate structural properties using thermodynamic information, we constructed a free energy landscape (FEL) for wiled type and mutant spike proteins (Fig. 4). On the FEL, the energy minima was obtained based on the probability of a combination of MD data points to map the minimum energy configuration of the proteins. As seen in Fig. 4, the red and yellow colors indicate lowest energy based on the stability of the spike protein. It can be said that according to the probability energy minima distribution, stability of S494P is higher than wild type.

3.4. Immunogenicity evaluation

At this stage, the immunogenicity of S494P mutation relative to wild type was obtained using MHC I binding prediction. Evaluation of the immunogenicity of mutant spike proteins indicates that they have different immunogenic responses. Based on the results, it can be said that P494 has a lower immunogenic capacity than the wild type (Table 4). Given that, increased stability can decrease the formation of aggregates and consequently, immunogenicity [38–40]; this may be why the immunogenicity of P494 is reduced.

Table 4
Average score
reported for T-cell
epitopes. The selected
length for MHC-I was
9.

sequence	MHC-I
S494	0.011
P494	0.005

3.5. Docking analysis

After running HADDOCK, the best cluster was selected according to the size of the cluster and its Z -score. To find out the affinity level of biological partner, total binding energy which is the sum of Van der Waals and Electrostatic energy is computed [41]. According to the results of docking analysis, the total binding energy of wild type and P494 spikes with their receptors were -339.8 and $-348.1 \text{ kcal mol}^{-1}$, respectively. Also, the Buried Surface Area (BSA), which measures the size of the interface in a protein–protein complex, in the spike^{P494} with its receptor is more than wild type complex (Table 5). The increase in BSA is due to a conformational change of spike^{P494} compared to the wild type and it can increase the chance of the interaction. Then HADDOCK results show better binding of spike^{P494} to its receptor relative to wild type complex.

For more details, changes in the residue binding patterns of wild type and mutant spike complexes were investigated by LigPlot (Fig. 5). Valuable information has been obtained about the spike-hACE2 molecular interactions. The type and number of some of the interface amino acids interactions are different. The number of Hbound in the wild type and spike^{P494} complexes are 15, and 16 respectively. Also, in the wild type, for example, the Ala475 forms one Hbound with Ser19 of hACE2, but do not have any role in interaction of mutant with hACE2. In addition, the Thr500 form two Hbounds with Tyr41 in the wild spike. However, on the spike^{P494} Thr500 forms one Hbound with Tyr41 and the other with Arg357. Moreover, Lys353 of hACE2 in complexes with spike^{P494} forms three Hbounds with Gln498, Gly496 and Asp501 residues, while in the interaction between wild spike, it forms two Hbounds with Gln498 and Gly496. Table 6 details the comparison of Hbounds between amino acids of spike-hACE2 complexes.

The effect of mutations on the binding pattern could be the result of structural changes in spike proteins through a new substitution. Figure 6 shows the structural changes related to area neighbor of mutant positions in the spike proteins. Comparison of physicochemical properties of wild and mutant residue showed that in the S494P mutation, Ser is the simplest hydroxyl amino acid and low side chain flexibility. On the other hand, free Pro is a secondary amine with the side chain curving back from the alpha carbon to bond to the amine nitrogen. When incorporated into a protein, Pro therefore lacks an amide proton and locks the phi backbone angle approximately -60° by the pyrrolidine ring and psi angles fall into two groupings near -45° and $+135^\circ$ in the helical and sheet structures. The backbone near a pro thus tends to be inflexible and may prevent certain secondary structures from forming.

Table 5

The statistics of the best clusters for native and mutant spike in interaction with hACE2.

Haddock criteria	Wild type	S494P
HADDOCK score	-154.3 +/- 3.9	-157.7 +/- 1.1
Cluster size	199	198
RMSD from the overall lowest-energy structure	0.7 +/- 0.5	0.7 +/- 0.4
Van der Waals energy	-68.8 +/- 4.0	-68.3 +/- 3.4
Electrostatic energy	-261.1 +/- 15.8	-272.2 +/- 11.8
Desolvation energy	-36.3 +/- 2.8	-32.0 +/- 2.7
Restrains violation energy	0.0 +/- 0.03	0.1 +/- 0.11
Buried Surface Area (Å ²)	1950.4 +/- 93.3	1976.0 +/- 68.9
Z-Score	0.0	0.0
Total Interaction energy	-339.8	-348.1

Table 6

Comparison of hydrogen bound between amino acids of spike-hACE2 complexes.

S ₁ hACE2	S ₁ hACE2	S ₁ hACE2
Ala475 (WT) Ser19	Tyr449 (WT) Asp38	Gly446 (WT) Gln42
Ala475 (S494P) -	Tyr449 (S494P) Asp38	Gly446 (S494P) Gln42
Lys417 (WT) Asp30	Tyr505 (WT) Asp38	Gly496 (WT) Lys353
Lys417 (S494P) Asp30	Tyr505 (S494P) -	Gly496 (S494P) Lys353
Gln493 (WT) Lys31	Gly496 (WT) Asp38	Gly496 (WT) Asp38
Gln493 (S494P) Lys31	Gly496 (S494P) -	Gly496 (S494P) -
Gln493 (WT) Glu35	Asn487 (WT) Tyr83	Tyr449 (WT) Gln42
Gln493 (S494P) Glu35	Asn487 (S494P) Tyr83	Tyr449 (S494P) Gln42
Thr500 ^a (WT) Tyr41	Asn487 (WT) Gln24	Lys353 (WT) -
Thr500 ^b (S494P) Tyr41	Asn487 (S494P) Gln24	Lys353 (S494P) Gly502
Gln498 (WT) Lys353	Tyr505 (WT) -	Asp501 (WT) -
Gln498 (S494P) Lys353	Tyr505 (S494P) Glu37	Asp501 (S494P) Lys353
Arg357 (WT) -		
Arg357 (S494P) Thr500		
^a two hydrogen bound, ^b one hydrogen bound.		

Conclusion

Although the new SARS-Cov-2 virus (COVID-19) has emerged since 2019, due to global pandemic, many studies are being done on this virus by researchers at the molecular level. In this study, all analyses were appropriate and convergent. Results from some of bioinformatics tools such as MD simulation, FoldX, PoPMuSiC and docking analysis showed increased stability and better interaction of mutant spike with its receptor. It means that S494P mutation results in structural changes in the mutant relative to the wild type structure that can increase binding affinity. Due to the fact that the P494 mutation in close contact with the hACE2 binding region, the enhanced binding affinity of P494 can be attributed to some factors such as conformational changes and then interfacial complementarity during hACE2 recognition and also the reorientation of some of residues at the interface which makes the number of hydrogen bonds more and stronger. Altogether, further S494P infection may be related to these conformational changes and new structural properties. Therefore, the use of other methods such as experimental stability analysis and S494P immunoassay studies to closely monitor about function, features, and other aspects of this SARS-COVID-19 mutation is important to find specific treatment in the next future.

Declarations

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Authors' contributions:

Mehr Ali Mahmood Janlou: Conception and design, Methodology, Data analysis and interpretation

Shadman shokravi: Conception and design , Writing—review and editing hassan sahebjaamee: Methodology, Visualization, Collection and assembly of data

All authors read and approved the final manuscript.

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Figures

Figure 1

A, Overall topology of the SARS-CoV-2 spike monomer. TD, topological domain; TM, transmembrane domain; TR, Tropism region; HP, heptad repeats; FP, fusion peptide; Cc, coiled coils; NTD, N-terminal domain. B, Overall structure of the SARS-CoV-2 RBD bound to ACE2. ACE2 is shown in green. The SARS-CoV-2 RBD core is shown in cyan and RBM in red. Disulfide bonds in the SARS-CoV-2 RBD are shown as sticks and indicated by arrows. The N-terminal helix of ACE2 responsible for binding is labeled.

Figure 2

A, Root-mean square deviation (RMSD); B, Root-mean-squared fluctuation (RMSF); and C, the Solvent accessible surface area (SASA) during MD simulation of native and mutant structures.

Figure 3

Average of secondary structures during the MD simulation of wild type and mutant spike proteins. Structure=A-Helix+B-Sheet+B-Bridge+Turn.

WT

P494

Figure 4

Projections of Free energy landscape of wild and mutant conformational space onto PC1 and PC2 produced from PCA of Desmond trajectories. The free energy landscape along the first two principal components PC1 and PC2 for (A) wild type and (B) S494P where red and yellow indicates energy minima configuration and blue shows the highest energy configuration.

Figure 5

Graphical representation of docking results. Figure panels from up to down represent the 2D interactions of Wild type, and P494 Spikes with hACE2, respectively.

S494 **P494** **Figure 6**

Displays the distance change between position 494 (S494 and P494) relative to other adjacent positions of the structures. Also, conversion of beta structure to loop is seen in P494.