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Research paper

Coevolutionary forces shaping the fitness of SARS-CoV-2 spike glycoprotein against human receptor ACE2

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ABSTRACT

The current global health problem caused by SARS-CoV-2 has challenged the scientific community in various ways. Therefore, worldwide several scientific groups are exploring SARS-CoV-2 from different aspects including its origin, spread, severe infectivity, and also to find a cure. It is now well known that spike glycoprotein helps SARS-CoV-2 to enter inside the human host through a cellular receptor ACE2. However, the role of coevolutionary forces that makes SARS-CoV-2 spike glycoprotein more fit towards its human host remains unexplored. Therefore, in present bioinformatics study we identify coevolving amino acids in spike glycoprotein. Additionally, the effects of coevolution on the stability of the spike glycoprotein as well as its binding with receptor ACE2 were predicted. The results clearly indicate that coevolutionary forces play a pivotal role in increasing the fitness of spike glycoprotein against ACE2.

1. Introduction

Coronaviruses are positive sense RNA viruses which cause various respiratory diseases in human ranging from common cold to severe infections such as SARS (Severe Acute Respiratory Syndrome), MERS (Middle East Respiratory Syndrome) and COVID-19 (Corona Virus Disease-2019). The COVID-19 is the recently emerged pandemic caused by SARS-CoV-2 which has infected more than 50,226,033 peoples and caused approximately 1,254,267 fatalities till 9 November 2020 (https://covid19.who.int/). Development of therapeutics is still under process and to control the ongoing pandemic of COVID-19 is an extreme challenge.

The survival and success of pathogen depends on its colonization capacity, by surpassing the defence mechanism of the host and reaching the appropriate niche. Entry of pathogen into the host cell is the first step of infection which also determines the impact of disease. Therefore, to know the ability of pathogen to enter inside the host cell remains a major concern for the researchers especially in the case of communicable diseases like COVID-19 that can cause a pandemic.

Four structural proteins, E (envelope protein), M (membrane protein), N (nucleo-capsid protein) and S (spike protein) are present in the SARS-CoV-2 virus while the entry of SARS-CoV-2 into human is facilitated by the interaction of spike protein with the host angiotensin-converting enzyme 2 (ACE2) similar to the entry of SARS-CoV (Tai et al., 2020; Wrapp et al., 2020). The spike protein is a trimeric class I fusion protein which allows the fusion of the viral membrane with the host receptor through structural rearrangements (Yan et al., 2020). The receptor binding domain (RBD) of spike glycoprotein allows the binding of SARS-CoV-2 with human receptor ACE2 and enables the entry of viral RNA into the human body (Tai et al., 2020; Wrapp et al., 2020). Apart from being the receptor of the SARS-CoV and SARS-CoV-2, ACE2 also regulate physiological roles like the regulation of Renin-Angiotensin system and facilitator of amino acids, therefore, it is widely expressed in cardiovascular tissues, gut, kidney, central nervous system and adipose tissue (Gheblawi et al., 2020). There is approximately 80% identity in the genome of SARS-CoV and SARS-CoV-2 (Xu et al., 2020). This raises the question that if the entry of SARS-CoV-2 is similar to SARS-CoV and there is high similarity at genomic level then which evolutionary factors are responsible to drive the higher infectivity in case of SARS-CoV-2.

Since coevolution of amino acids plays an important role in the stability and interaction pattern of the protein (Chakrabarti and Panchenko, 2010), therefore, it is also necessary to understand the coevolutionary pattern of the spike protein. Coevolution helps to fix a mutation of one amino acid with compensatory mutation of another amino acid or groups of amino acids (Vats and Shanker, 2019).

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Moreover, it suggests which amino acids work under evolutionary pressure to make a protein fit for its environment.

Generally, the binding pocket of a protein has been considered as a functional determinant. However, in many cases the residues which were not the part of a binding domain also played an important role in determining the functional properties of a protein by controlling stability, intrinsic motion or disturbing the binding affinity with receptor molecules (Bollen et al., 2012; Follis et al., 2018; Priya et al., 2017). The identification of such crucial amino acids is a challenging task and also of utmost importance. Here, we consider the role of coevolution to identify the group of amino acids which may act as a structural and/or functional determinant in spike glycoprotein. The motivation behind this idea is that evolution holds the key to COVID-19 like pandemic, which happens once in a century of infectious cycles and certainly leaves the footprint for the adaptation of pathogens to a new host, i.e., humans in this case. Considering this, the present study was planned to detect coevolving groups in spike protein of SARS-CoV-2. The identified coevolving groups were used to check the effect on structure of spike protein and its affinity with ACE2.

2. Materials and methods

2.1. Detection of coevolving groups in spike protein of SARS-CoV-2

Sequences of spike glycoprotein corresponding to NCBI GenBank accession ids MN908947 (Human SARS-CoV-2), MN996532 (Bat coronavirus RaTG13), AY278741 (SARS coronavirus Urbani), KY417146 (Bat SARS-like coronavirus) and MK211376 (Coronavirus BtRs-BetaCoV/YN2018B) were used for the analysis. These sequences were used recently to shed light on the natural or purposeful manipulated origin of SARS-CoV-2 (Andersen et al., 2020).

The multiple sequence alignment (MSA; supplementary file 1: SF1) of protein sequences was produced using default parameters of MAFFT (Katoh and Standley, 2013). Phylogenetic tree of MSA was inferred with PhyML (Guindon et al., 2010) and used for coevolution detection as implemented in CoMap v1.5.2 program (Dutheil et al., 2005). Amino acids were weighted based on biochemical properties namely Grantham, polarity, charge, and volume. The statistical significance (p-value $\leq$0.05) of identified coevolving sites and false discovery rate were evaluated. Circular plot was created to show coevolving positions with the help of Circos (Krzywinski et al., 2009). Each coevolving amino acid residue in MSA was masked to its residue class (**Residue Mask**: Amino acid; **A**: D, E; **B**: R, K; **G**: G; **N**: A, L, I, V, M; **P**: P; **Q**: S, T, C, N, Q; **R**: F, Y, W, H) as done earlier (Hecht et al., 2011; Shanker, 2016; Vats and Shanker, 2019).

2.2. Structural analysis of the coevolving groups in spike protein of SARS-CoV-2

The identified coevolving amino acids were mapped to their secondary structure (alpha helix: H or beta-sheet: S) considering Protein Data Bank file having PDB ID 6VSB (Wrapp et al., 2020). The missing residues were built using CHARMM-GUI web-server (Jo et al., 2008). The effect of amino acids coevolution on the stability of spike protein was examined by introducing reverse mutations using DynaMut web server, which analyses the effect of point mutation on protein dynamics and stability (Rodrigues et al., 2018). To check the impact of coevolving amino acids on spike protein stability, first the effect of point mutation was checked for one amino acid and the mutated PDB was used as an input to know the effect of another mutation in the same coevolving group (Fig. S1).

To identify the coevolution induced changes in the interaction pattern of spike protein with the human receptor, docking between spike glycoprotein and ACE2, PDB ID 2AJF (Li et al., 2005) was performed by HADDOCK protein-protein docking server (de Vries et al., 2010). Moreover, mutation induced changes in binding affinity of spike glycoprotein with ACE2 were predicted by mCSM web-server (Pires et al., 2014b).

3. Results and discussion

3.1. Coevolving groups in spike protein of SARS-CoV-2

The coevolving groups were detected in spike protein of SARS-CoV-2 using homologous sequences. A total of 99 coevolving groups were detected (Fig. 1 and File SF2). Among these, the amino acids were found in groups of 2, 3, 4 and 6. Considering complete sequence, 148 (11.63%) out of 1273 amino acids were involved in coevolution in spike protein of SARS-CoV-2. Among identified groups, 47 coevolving amino acids were found with secondary structure (16 Helix and 31 Sheets). Some of the coevolving groups detected along with their amino acid residue class and secondary structure are given in Table 1. This table shows the coevolving sites (positions) in MSA along with their amino acids, residue class of amino acids in bold and secondary structural state (Helix or Sheet) of amino acids. Additionally, the MSA of sequences used in analysis (supplementary file 1: SF1), a complete list of identified coevolving groups (SF2), their amino acid residue class (SF3), and groups with the involvement of secondary structure along with their relative (after MSA) and absolute (before MSA) position of amino acids (SF4) are provided. The files SF2 and SF3 differ only for amino acids and their residue class at coevolving sites. The residue class helps to know the conservative mutation which changes an amino acid to another amino acid with similar biochemical properties at a site. Since conserved residues are usually preferred as epitopes for vaccine development (Ahmed et al., 2020) as well as targets for drugs, the amino acid residue class will assist in selecting residues with similar biochemical properties while considering variability at a site to construct variable epitope libraries (Servin-Blanco et al., 2018).

The coevolving groups were detected in spike protein of SARS-CoV-2 using homologous sequences evolved from common ancestor. The coevolution in several amino acids (148; 11.63%) of spike protein (1273 amino acids) of SARS-CoV-2 spread throughout the sequence.

3.2. Coevolution directed structural stabilization of spike protein

To check the effects of coevolution on the stability of spike protein, the structure with PDB ID 6VSB was used with amino acid residues number 27 to 1146.The missing intermediate residues were built using CHARMM-GUI web-server (Fig. 2a). The coevolving groups may play direct considerable effects on the structure and functional activity of the spike protein. Therefore, we analysed four coevolving groups (SF2 group numbers 6, 14, 26 and 57) with presence of at least one amino acid in the RBD. The positions of amino acids mentioned in SF2 are the relative positions (after alignment generation in which coevolution was detected) which differ from their absolute positions (without alignment). To avoid any confusion the respective absolute positions of the selected four groups are mentioned in Table S1. The distance among the amino acid residues of group number 57 is shown in Fig. 2b, which clearly indicates that these residues are spatially distant in protein structure but still work under evolutionary pressure and constitute a coevolving group. It has already been demonstrated that amino acid residues especially in homo-oligomeric interfaces have a tendency to coevolve even if they are more than 15 Å apart in protein structures (Anishchenko et al., 2017).

The effect of the individual mutations on the stability of three dimensional structure was analysed for reverse mutant (change from SARS-CoV-2 to SARS-CoV) of four coevolving groups (Table S1) using the structure of spike protein of SARS-CoV-2 and the results were then interpreted to know the effect of coevolution from SARS-CoV to SARS-CoV-2. To find the overall effect of the coevolving group on the structure of spike protein, the effect of each mutation was summed up. The results of structural stability analysis from DynaMut web-server showed that the reverse mutations of these coevolving residues destabilize the

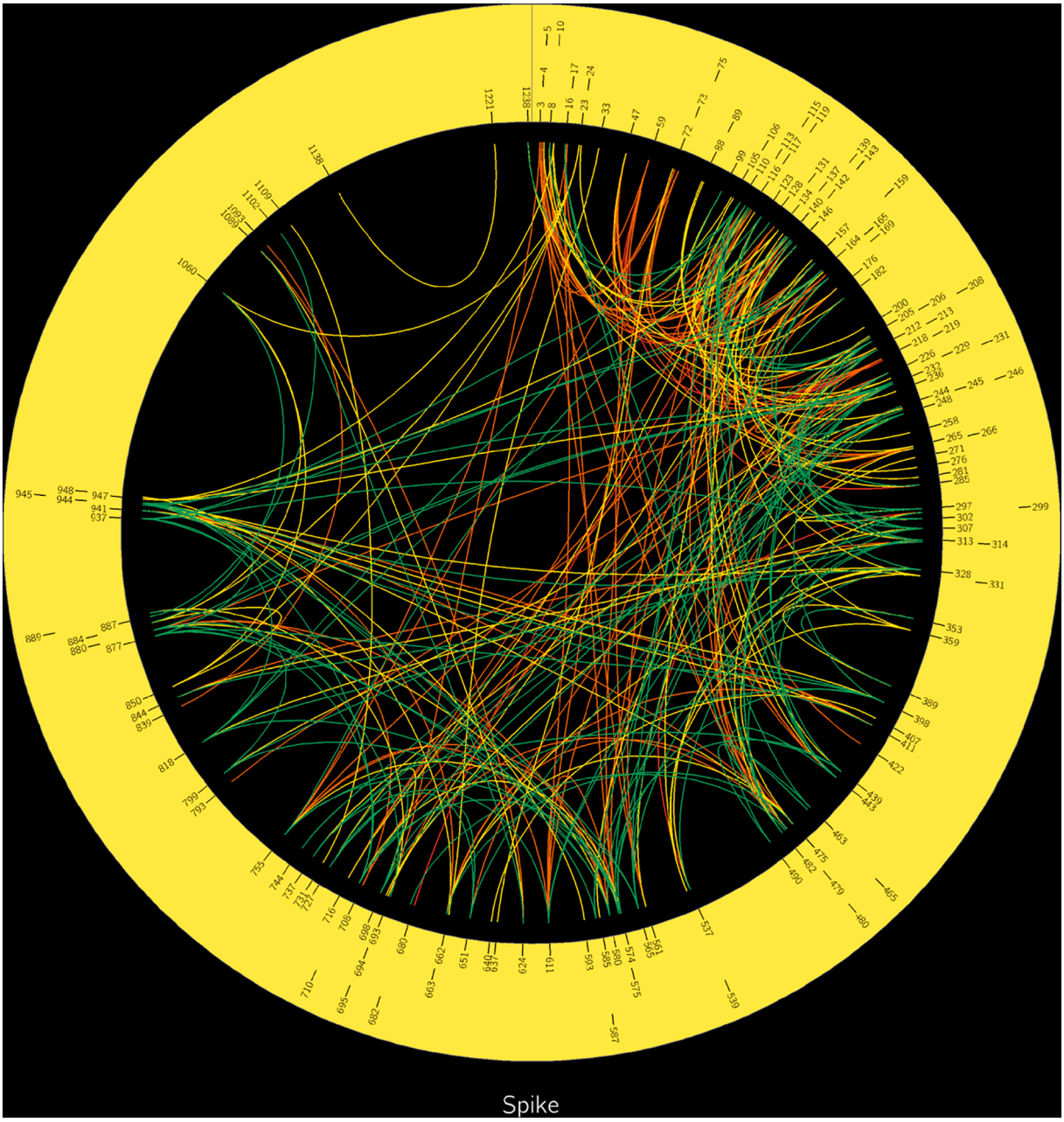

Fig. 1. The groups of coevolving amino acids detected in the spike protein of SARS-CoV-2. The outer yellow circle depicts the relative positions of amino acids detected as part of coevolving groups in the multiple sequence alignment. The colored links show the groups of coevolving amino acids detected by weighted substitutions namely Grantham (Orange), polarity (Yellow), volume (Green), and charge (Red).

structure (Table 2, S2–S4). In these tables, the negative values reflect the destabilization of the structure (Rodrigues et al., 2018) and the structure destabilization of the reverse mutant (change from SARS-CoV-2 to SARS-CoV) was interpreted as a stabilizing effect for forward mutation (from SARS-CoV to SARS-CoV-2). The effect of an evolutionary destabilizing change of one amino acid may be compensated by another mutation without having direct physical contact between them (Pazos and Valencia, 2008). Therefore, the overall effect can only be considered as the sum of all the steps involved in generating reverse mutant, which is destabilization in all four coevolving groups considered for structure analysis. Since SARS-CoV-2 infection in humans is a recent phenomenon, therefore, we have reverse mutated the spike protein at selected coevolving positions considering homologous characters of AY278741 (SARS coronavirus Urbani), KY417146 (Bat SARS-like coronavirus), and MK211376 (Coronavirus BtRs-BetaCoV/YN2018B) to check the stability of structure which turns out to be destabilizing and points to stability in spike protein of SARS-CoV-2. It clearly indicates that during the evolution of SARS-CoV-2 to infect humans, nature have induced selective mutations in group with a tendency to stabilize the three dimensional structure of the spike protein. These results were also confirmed by ENCoM (Frappier et al., 2015), mCSM (Pires et al., 2014b), SDM (Pandurangan et al., 2017), and DUET (Pires et al., 2014a) servers which differs in their calculation methodology. Therefore, the results of structural analysis clearly indicate that reverse mutations in these four coevolving groups destabilized the structure of spike glycoprotein when changed from SARS-CoV-2 to SARS-CoV, hence, stabilized in the case of forward mutation. The mutations which provide stability to proteins have the higher chance to remain in a population and if changes are responsible for a disease it may lead to catastrophe. It is difficult to regulate the evolutionary successful events, which may be one of the

Table 1
Some of the coevolving groups detected along with their amino acid residue class (**in bold**) and secondary structure information.

S. No. in SF2[#]	Coevolving Groups	Size	p-value	Weight[^]	FDR[*]
4.	H/L 59; R/S 219; D/N 116; D/N 142 **N/R 59; B/Q 219; A/Q 116; A/Q 142** [&]Site-59 (54); Sheet; amino acid L	4	0.008	Charge	yes
5.	S/T 119; S/T 716; F/L 146; F/L 206 **Q 119; Q 716; N/R 146; N/R 206** Site-716 (711); Sheet; amino acid S Site-206 (201); Sheet; amino acid F	4	0.009	Grantham	yes
57.	E/N 137; E/N 359; I/V 331; I/V 539 **A/Q 137; A/Q 359; N 331; N 539** Site-359 (354); Sheet; amino acid N	4	0.003	Polarity	yes
67.	S/T 328; S/T 944; N/T 475; N/T 663 **Q 328; Q 944; Q 475; Q 663** Site-944 (939); Helix; amino acid S	4	0.000	Polarity	yes
74.	F/L 146; F/L 206 **N/R 146; N/R 206** Site-206 (201); Sheet; amino acid F	2	0.004	Volume	yes

[^] Biochemical property used for coevolution analysis.
[*] FDR – False Discovery Rate.
[&] Site-Relative position after MSA (Absolute position before MSA).
[#] S. No. of coevolving groups as given in SF2.

strongest reasons for the current COVID-19 pandemic scenario the world is facing.

3.3. Coevolution induced changes in the interaction pattern of spike protein with ACE2

The structure of spike protein was docked with the crystal structure of ACE2 (PDB ID 2AJF) to check the effect of coevolution on the binding affinity of spike protein with the human receptor (Li et al., 2005). The docking was performed as implemented in HADDOCK using restrained F486, L455, Q493, S494 and N501 of spike glycoprotein, whereas M82, K31, E35, D38 and K353 of ACE2, which were found important in the binding (Andersen et al., 2020). The top most structure was selected from the cluster with the highest dock score, (Fig. 3a) and considered for further analysis (Table S5). To check the reliability of the docked conformation it was superimposed on the available incomplete crystal structure of the bound complex (Lan et al., 2020). The rmsd of the docked conformation of C-alpha atoms and all atoms are 0.579 Å and

Table 2
Change in the stability of spike protein of SARS-CoV-2 induced by the reverse mutations of coevolving residues in group 57. The mutation used for stability analysis is given in bold. The data shown here is in kcal/mol.

Protein stability	Web-servers used to predict structural stability of spike protein				
	DynaMut	ENCoM	mCSM	SDM	DUET
$\Delta\Delta G_1$ (**E132N**)	0.136	−0.445	0.516	−0.1	0.666
$\Delta\Delta G_2$ (E132N + **I326V**)	−1.51	−0.377	−1.488	−2.81	−1.914
$\Delta\Delta G_3$ (E132N + I326V + **N354E**)	0.454	0.028	−0.327	0.57	0.23
$\Delta\Delta G_4$ (E132N + I326V + N354E + **V534I**)	0.101	0.092	−0.574	0.13	−0.234
ΔΔG (Reverse mutant of SARS-CoV-2 spike protein)	**−0.819**	**−0.702**	**−1.873**	**−2.21**	**−1.258**

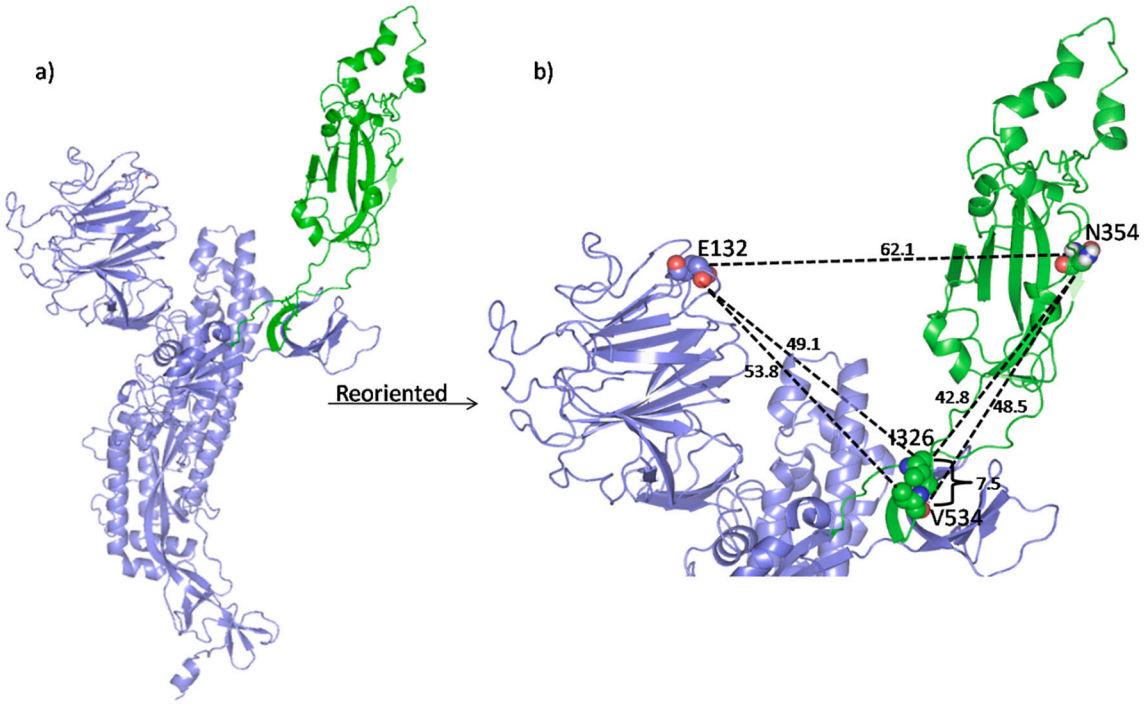

Fig. 2. a) Structure of the pre-fusion 2019-nCov spike glycoprotein; image was generated after building missing intermediate residues of PDB ID: 6VSB as mentioned in the method section. The RBD of the spike protein is represented in green and the rest of the protein in blue. b) The reoriented zoom-in picture of spike glycoprotein showing the coevolving residues of group 57, which also involves the RBD. The distances among C-alpha atoms of the coevolving residues are shown in angstrom (E132-N354: 62.1 Å; N354-I326: 42.8 Å; N354-V534: 48.5 Å; V534-I326: 7.5 Å; V534-E132: 53.8 Å; I326-E132: 49.1 Å).

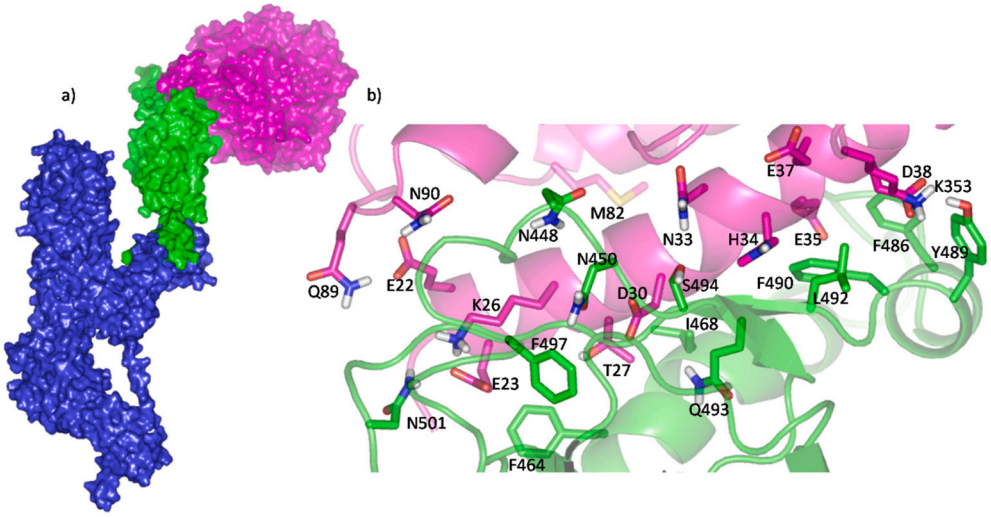

Fig. 3. a) Surface representation of SARS-CoV-2 spike glycoprotein's RBD (green) docked with ACE2 (magenta). b) The side chains of major interacting residues are represented as sticks.

0.691 Å, respectively (Fig. S2). The lower rmsd values substantiate the acceptability of the docked conformation for further analyses.

A close inspection revealed that the compactness of the interaction involves many polar residues. The amino acids F486, Y489, F490, L492, F497, N448, N501, I468, F464 of spike protein and D38, E35, N33, M82, K26, E22, K353 of ACE2 were observed as the major interacting residues present at the interface (Fig. 3b).

The four selected coevolving groups were also analysed by mCSM web-server to check the effect of mutations on the binding affinity of spike glycoprotein with ACE2; interestingly, the results showed the reduced affinity for each group of mutations (Table 3 and Table S6). This was also confirmed by the docking between spike glycoprotein with the reverse mutated coevolving residues and ACE2 which showed lower affinity (Table S5). These results indicate the higher infectious potency of SARS-CoV-2 spike protein as compared with SARS-CoV.

Furthermore, group 57 (Table S1; E132N, I326V, N354E, and V534I) was selected for detailed atomistic level structural analysis. This group includes three positions (I326V, N354E and V534I) from the RBD of the spike glycoprotein (Lan et al., 2020). The importance of N354 was recently reported, where it was found as a member of a higher affinity group in the binding of spike protein with ACE2, and provides a stable structure of RBD/ACE2 with increased infectivity of the SARS-CoV-2 (Ou et al., 2020). The all atom RMSD between spike protein of wild-type docked complex of SARS-CoV-2 with ACE2, and docked complex with reverse mutant of coevolving group number 57 is 0.93 Å.

In docked complex with reverse mutant of coevolving group number 57, the side chain reorientation in K31 of ACE2 while N487 and R457 of spike protein reduced the compactness at the binding interface (Fig. 4a and b). In reverse mutant the distance between side chain N-atoms of N487 in spike protein and K31 of ACE2 has increased by 4.4 Å whereas the distance between C-alpha atoms between these residues increased by 1.7 Å (Fig. 5a and b). The change in the distances between these atoms was driven by the reorientation of N487 and K31. It was also influenced by the presence of negatively charged amino acid E35 of ACE2 and E471 of spike protein at the binding site (Fig. 4). In SARS-CoV-2 and ACE2 complex the K31 of ACE2 interacts with E471 of spike protein and the side-chain nitrogen atom of N471 orient towards the binding interface (distance between side chain H-atom of K31 and side chain O-atom of E471 is 1.6 Å in SARS-CoV-2 while 2.6 Å in case of reverse mutant), however, in reverse mutated complex, K31 moved towards E35 of ACE2 (distance between side chain H-atom of K31 and side chain O-atom of E35 is 4.3 Å in SARS-CoV-2 while 1.6 Å in case of reverse mutant) and the side chain nitrogen atom of N487 flipped away from the complex interface (Fig. 4). This confirms the loss of compactness in case of reverse mutant complex with ACE2 which indicates that forward mutation induced the compactness and consequently improved the binding affinity of SARS-CoV-2 spike protein with human ACE2. The reverse mutant induced reduction in electrostatic and Van der Waals energy and the overall docked score also indicates the same (Table S5). These results reflect that the spike protein of SARS-CoV-2 is in a highly stable state and binds to the ACE2 with the higher affinity as compared to the reverse mutant.

Table 3
Change in the binding affinity of spike protein-ACE2 complex induced by the mutations of coevolving group 57. Negative values represent a decrease in the binding affinity.

S. No. in SF2[#]	Mutation	ΔΔG (kcal/mol)	Change in spike protein-ACE2 complex affinity
57.	E132N	−0.689	Decrease
	I326V	−0.062	Decrease
	N354E	−0.859	Decrease
	V534I	−0.82	Decrease

[#] S. No. of coevolving groups as given in SF2.

4. Conclusions

The findings of present coevolutionary analysis depicts that coevolving groups are providing improved binding affinity to make SARS-CoV-2 infection more successful in humans. As sequence determines the structure of a protein, it can be argued that during evolution over years the spike glycoprotein has fine-tuned itself to attain more affinity and stability in infecting humans. The effects of coevolving groups on the stability, affinity and interaction patterns of the spike protein of SARS-CoV-2 indicate the coevolution mediated structural and functional regulation of the protein. The approach followed here can also be used as a model study to understand infectivity of other dreadful pathogens.

Author contributions

AS conceived the study. PP and AS performed computational analyses, analysed the data and wrote the MS.

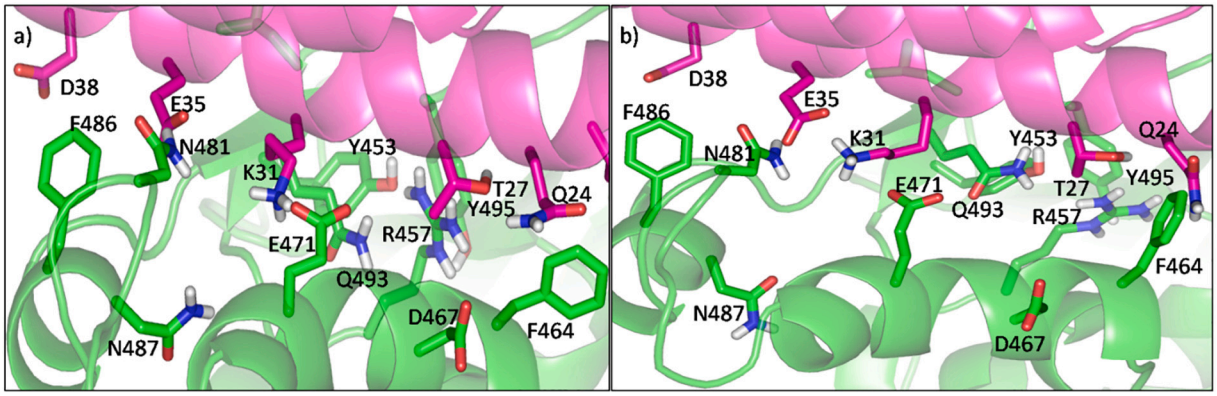

Fig. 4. The interacting residues in the docked structure of ACE2 (magenta) with a) RBD of spike protein (green) of SARS-CoV-2. b) Reverse mutated structure of spike protein considering coevolving group 57. The reorientation of the side chains of K31 in ACE2, whereas N487 and R457 of spike protein reduce the compactness at the binding interface. That has also been reflected in the docked score.

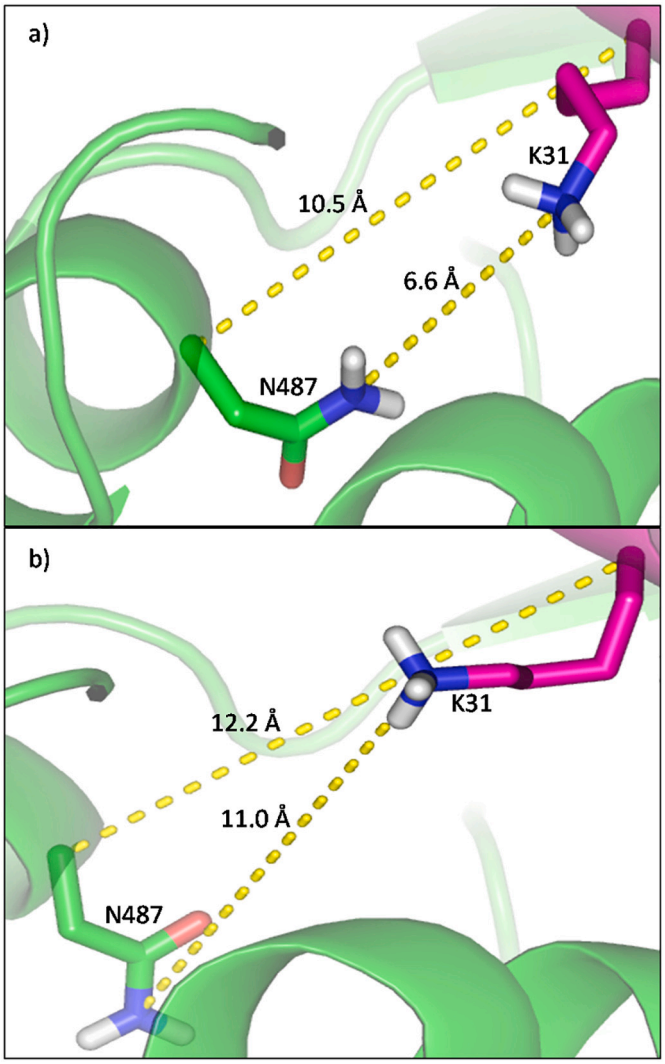

Fig. 5. The distance between N487 of RBD (green) and K31 of ACE2 (magenta) in a) SARS-CoV-2 (CA_{N487}-CA_{K31}: 10.5 Å; Side-chain N_{N487}-Side-chain N_{K31}: 6.6 Å). b) Reverse mutant considering coevolving group 57 (CA_{N487}-CA_{K31}: 12.2 Å; Side-chain N_{N487}-Side-chain N_{K31}: 11.0 Å).

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Declaration of Competing Interest

None.

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