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Medicine in Drug Discovery 13 (2022) 100114

Contents lists available at ScienceDirect

Medicine in Drug Discovery

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

Short Communication

The Delta variant mutations in the receptor binding domain of SARS-CoV-2 show enhanced electrostatic interactions with the ACE2

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ARTICLE INFO

Keywords:
SARS-CoV-2
Binding energy
Protein–protein interactions
Electrostatic interactions
Monte Carlo

ABSTRACT

The mutations in the receptor binding domain (RBD) of the SARS-CoV-2 are shown to enhance its replication, transmissibility, and binding to host cells. Recently, a new strain is reported in India that includes mutations (T478K, and L452R) in the RBD, which are possibly increasing the infection rate. Here, using Molecular Mechanics (MM) and Monte Carlo (MC) sampling, we show that the mutations in the RBD of the Delta variant of SARS-CoV-2 induced conformational changes in ACE2-E37, which enhanced the electrostatic interactions by the formation of a salt-bridge with SARS-CoV-2-R403. In addition, we observed that these mutations altered the electrostatic interactions of the salt-bridge formed between the RBD-T500 and the ACE2-D355, which reduced by more than 70% compared the to the WT.

The Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) belongs to the large family of viruses that were reported to cause respiratory diseases in humans called Corona viruses (CoVs) [1]. Coronaviruses family have caused two previous severe pandemics: SARS and MERS in 2003 and 2012 [1]. SARS-CoV-2 is the virus that was reported to cause the novel COVID-19 pandemic. In December 2019, a cluster of severe unidentified pneumonia disease have been reported in Wuhan, China. In January 2020, COVID-19 outbreak emerged substantially and explosively worldwide till it was officially considered as a pandemic by the WHO. As of July 2021, the total number of confirmed COVID-19 cases have accumulated to over 197 million cases while the death toll was escalated to over 4 million deaths affecting more than 180 countries [1–6]. The angiotensin converting enzyme 2 (ACE2) is the target protein in humans (hACE2) and many other species that mediates the cell entry via binding with the SARS-CoV-2 spike protein [4,7–9]. Like all other viruses, SARS-CoV-2 exhibits changes all the time. Some changes may affect the viruses' properties "severity, spreading rate, …etc. while others may have no effect at all. It was recently reported that the mutations in the virus have led to an increase in the virus transmissibility due to change in the receptor-binding domain (RBD) or in the furin cleavage site [10–13]. There are four variants of concern (VOC) so far; alpha, Beta, Gamma, and Delta have been emerged out of the original virus [14]. The Delta variant has mutations in the S protein that are suggested to affect the virus transmissibility alongside its susceptibility to be neutralized by the previous COVID-19 variants' antibodies [15–17]. Those changes include two mutations in the RBD; Leucine at position 452 to Arginine (L452R) and Threonine at position 478 to Lysine (T478K) [16,17]. The mutation L452R have been reported to enhance the binding affinity to the host entry receptor ACE2, transmissibility, fitness, and infectivity and so improves the viral replication [18,19]. Besides, L452R mutation recently reported to enhance the viral replication by increasing the S protein stability and viral infectivity and viral fusogenicity [19,20]. The infectivity of the virus is mainly affected by different protein–protein interactions [21]. Mutations occur in the vital residues at the binding site would alter the adhesion of virus to host cells [20]. In this research, we study the effect of both of L452R and T478K variants in the RBD of the SARS-CoV2 on its binding to ACE2. The electrostatic interactions are known to be dominant over various protein–protein interactions [15]. Therefore, we used both of Molecular mechanics **MM** and Monte Carlo **MC** simulations to evaluate the interactions between RBD of S-protein and ACE2 at molecular level. We performed

Abbreviations: SARS, Severe acute respiratory syndrome; MERS, Middle East Respiratory Syndrome; WHO, World Health Organization; ACE2, Angiotensin-Converting Enzyme 2; *h*ACE2, Human Angiotensin-Converting Enzyme 2; RBD, Receptor Binding Domain; VOC, Variants Of Concern; WT, Wild Type; vdW, Van der Waals.

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https://doi.org/10.1016/j.medidd.2021.100114
Received 2 November 2021; Revised 14 November 2021; Accepted 22 November 2021
Available online 4 December 2021

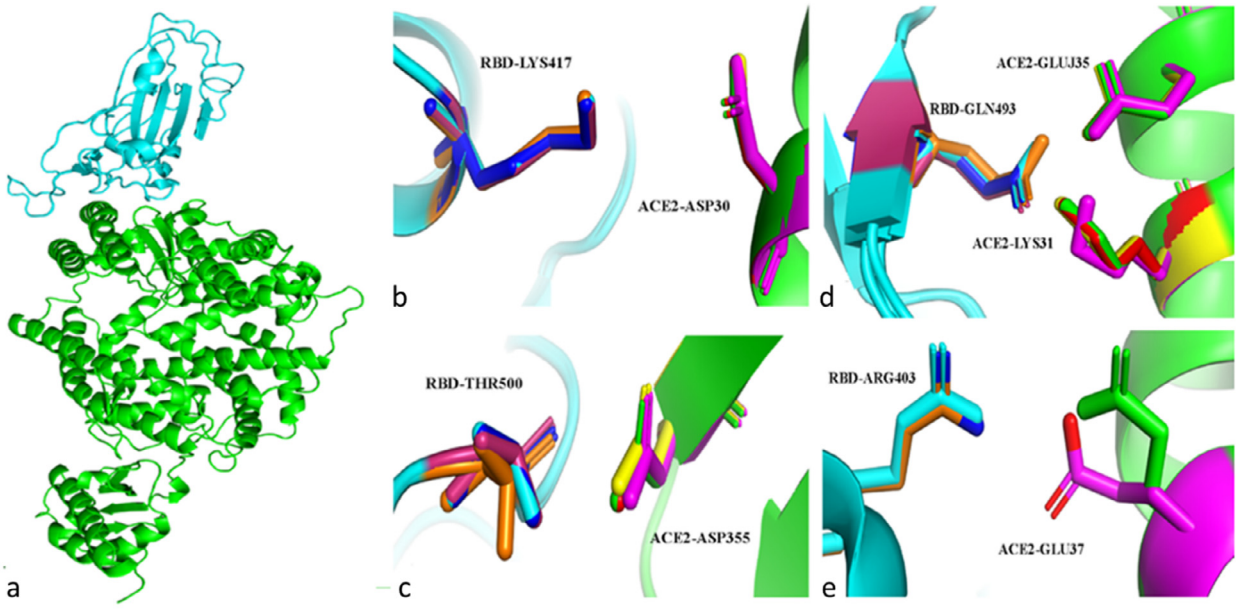

Fig. 1. a. The SARS-CoV-2 RBD protein (cyan) with ACE2 protein (green). b–e. The interactions between selected residues at the RBD/ACE2 interface in the WT protein and the structures with a single RBD mutation (L452R or T478K) and double mutation (L452R and T478K). The WT RBD and ACE2 are presented in cyan and green, respectively. RBDs of L452R, or T478K mutated structures are shown in Pink and Blue respectively, while ACE2 associated with these structures are shown in Yellow and Red, respectively. Finally, the double mutated (L452R and T478K) structure is presented in Orange and Magenta for RBD and ACE2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

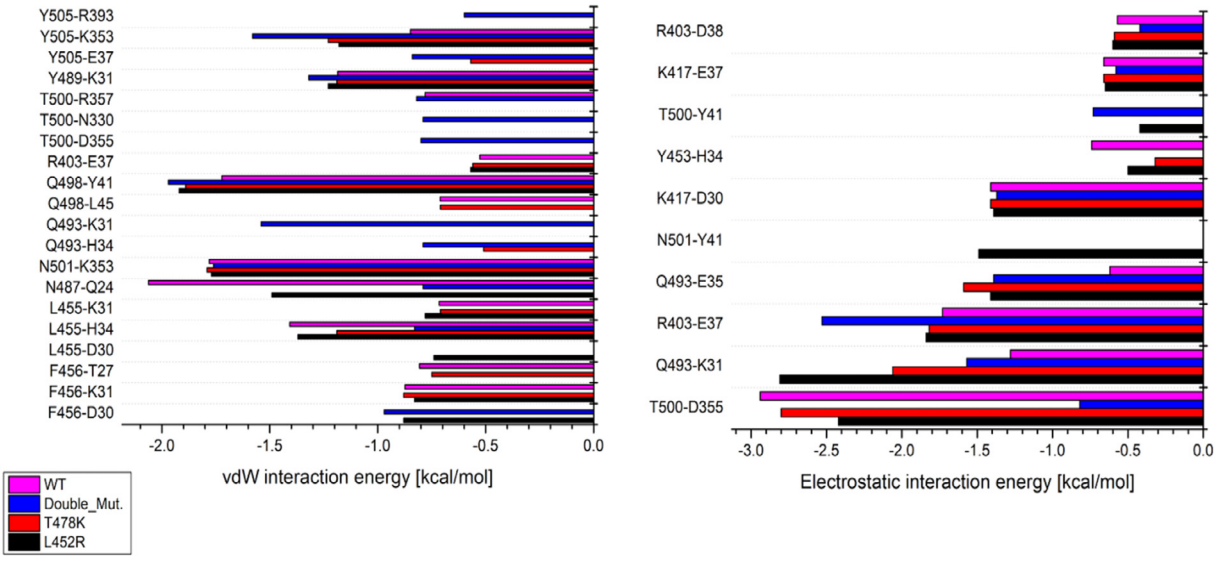

Fig. 2. Selected favorable vdW and Electrostatic interactions between residues at the RBD/ACE2 binding interface. The x-axis is the interaction energies (Energy in kcal/mol), while y-axis reflects the selected pairs of residues at the RBD/ACE2 interface. left and right panels: Depiction of vdW and electrostatic interaction energies, respectively, associated with each structure (wild type (magenta), T478K mutated structure (red), L452R mutated structure (black), and structures with double mutations (L452R and T478K) (blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
The interaction energies between SARS-CoV-2-RBD and ACE2 in WT, single mutated proteins L452R and T478K, and protein structure with T478K and L452R mutations combined.

	Coulomb	Van der Waals	Total
T478K and L452R	−7.98	−38.69	−46.67
L452R	−9.98	−32.03	−42.01
WT	**−6.64**	**−32.74**	**−39.38**
T478K	−6.09	−32.79	−38.88

our calculations for the aforementioned mutations and compared our results with the wild type (Native) protein. All calculations were based on the protein structure retrieved from the protein data bank (PDB ID: 6m17). Firstly, the crystal structure was optimized using openMM [22]. Followed by the generation of rotamers using MCCE [23], where that each rotatable bond was rotated by 60° to, properly, sample the sidechains' conformations. To build both variants L452R and T478K, the sidechains of L452 and T478 were replaced by sidechains of Arginine and Lysine, respectively, using MCCE. Finally, optimized protein

structures with the most occupied conformers were used to calculate electrostatic interactions by solving Poisson Boltzmann equation using DELPHI [24]. Then, Boltzmann distribution for all conformers is computed at pH 7 using MCCE for the WT and the mutated structures. The contribution of the electrostatic and the van der Waals (vdW) forces to the interaction energies of SARS-CoV-2/ACE2 were calculated for the single (L452R or T478K) and the double (L452R and T478K) mutated structures as well as the WT protein. In contrary to T478K, the L452R variant is shown to enhance the total binding energy between RBD and ACE2 by 2.63 kcal/mol compared to the WT protein. Furthermore, the interactions in the structure with both mutations L452R and T478K are enhanced by ~7.29 kcal/mol compared to WT protein see Table 1.

For WT protein, vdW interaction is shown to be maximum between RBD-N487 and ACE2-Q24 with ~−2.06 kcal/mol. The L451R and T478K mutations in the RBD has noticeable effect on the vdW interactions between the RBD-N487 and ACE2-Q24, which decreased by ~1.27 Kcal/mol. Moreover, the maximum vdW interactions are observed between RBD-Q498 and ACE2-Y41 in the structure with both mutations T478K and L452R (~−1.97 kcal/mol) and structures with single mutation T478K (−1.89 kcal/mol) or L452R (−1.92 kcal/mol). Based on our calculations, the formation of RDB-R403/ACE2-D30, RBD-K417/ACE2-D30, RBD-K417/ACE2-E37, and RBD-R403/ACE2-D38 salt bridges were conserved among all structures with interaction energies of ~−0.4, −1.4, −0.6 and −0.4 kcal/mol, respectively. The salt bridge between RBD-R403 and ACE2-E35 is observed in the mutated structure with electrostatic interaction energies of about −0.4 kcal/mol. Our data demonstrated that all mutations enhanced the electrostatic interactions between RBD-Q493 and ACE2-K31, where that the mutation L452R induced a maximum increase in the electrostatic interactions of about −1.53 kcal/mol compared to its value in WT protein. In WT and in the structure with single mutation T478K, maximum electrostatic interactions were reported between RBD-T500 and ACE2-D355 of ~−2.94 and −2.8 kcal/mol, respectively. These interactions are shown to be, slightly, reduced by the L452R mutation (0.52 kcal/mol more positive compared to WT protein). For the structure with both mutations T478K and L452R, the RBD-T500 and ACE2-D355 exhibit less attraction than in WT by ~−2.12 kcal/mol. In contrary to structures with single mutation, the structure with double mutations (T478K and L452R) in RBD is shown to induce a significant conformational change in sidechain of ACE2-E37 residue, Fig. 1. Structures with single mutation T478K or L452R induced an increase in the electrostatic interactions between RBD-R403 and ACE2-E37 by ~0.1 kcal/mol, while in the structure with the T478K and L452R mutations, the interaction energies of the salt bridge RBD-R403 and ACE2-E37 increased by ~0.8 kcal/mol compared to the WT Fig. 2.

Herein, we showed that the binding affinity of SARS-CoV-2 to human ACE2 is higher in the double mutated structure (T478K and L452R) than that in WT because of the significant changes in the electrostatic and vdW interactions. Our simulations show an enhanced electrostatic interactions between the E37-ACE2 and the R403-RBD aminoacids due to the delta variant mutations. Moreover, the salt-bridge electrostatic interactions between the RBD-T500 and the ACE2-D355 decreased compared to the WT protein.

Conflicts of interest

Authors declare that there are no conflicts of interest.

CRediT authorship contribution statement

Shaimaa S. Goher: Formal analysis, Writing. **Fedaa Ali:** Formal analysis, Writing. **Muhamed Amin:** Conceptualization, Supervision.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.medidd.2021.100114.

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