Computational Insights into the Conformational Accessibility and Binding Strength of SARS-CoV-2 Spike Protein to Human Angiotensin-Converting Enzyme 2

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ABSTRACT: The spike protein of SARS-CoV-2 (CoV-2-S) mediates the virus entry into human cells. Experimental studies have shown the stronger binding affinity of the RBD (receptor binding domain) of CoV-2-S to angiotensin-converting enzyme 2 (ACE2) as compared to that of SARS-CoV spike (CoV−S). However, a similar or weaker binding affinity of CoV-2-S compared to that of CoV−S is observed if entire spikes are used in the bioassay. To explore the underlying mechanism, we calculated the binding affinities of the RBDs to ACE2 and simulated the transitions between ACE2-inaccessible and -accessible conformations. We found that the ACE2-accessible angle of CoV-2-S is 52.2° and that the ACE2 binding strength of CoV-2-S RBD is much stronger than that of CoV−S RBD. However, CoV-2-S has much less of an ACE2-accessible conformation and is much more difficult to shift from ACE2-inaccessible to -accessible than CoV−S, making the binding affinity of the entire protein decrease. Further analysis revealed key interactional residues for strong binding and five potential ligand-binding pockets for drug research.

A new coronavirus known as SARS-CoV-2 has emerged and spread worldwide rapidly.1−4 Although the number of infections is still growing quickly, very few therapies have shown promising results.5−7 The spike (S) glycoprotein of SARS-CoV-2 (CoV-2-S hereinafter) plays an essential role in viral infection, engaging human angiotensin-converting enzyme 2 (ACE2) as a receptor and mediating the fusion of SARS-CoV-2 (SARS-CoV-2 hereinafter) with cellular membranes,8−9 making it an ideal target for developing drugs against SARS-CoV-2. To expose enough space for ACE2 binding, the receptor-binding domain (RBD) of CoV-2-S undergoes hinge-like structural changes from “down” to “up” states. The RBD down conformation (small RBD angle defined as the angle of D405−V622−V991, Figure S1) is a receptor-inaccessible state, and the RBD up conformation (large RBD angle) is a receptor-accessible state.

Recently, a number of biophysical and simulation studies have revealed the higher affinity of CoV-2-S RBD binding to ACE2 as compared to that of CoV−S RBD.10−16 However, it was found that the ACE2 binding affinity of the entire CoV-2-S is lower than that of CoV−S in a protein pull-down assay.17 Another study using a Blitz assay revealed that the ACE2 binding affinity of entire CoV-2-S is similar to that of CoV−S.18 The results with the entire protein presented a contradictory finding from the observations based on the RBD domains. Accordingly, there are several questions that remain unanswered. While the structural integrity of the spikes has a significant effect on the bioassay of the ACE2 binding affinity, the mechanism of this effect is unknown.

Several structures with different RBD up states of CoV-2-S are available in the Protein Data Bank (PDB),19−25 but there is no criterion defined to judge whether a conformation is accessible or inaccessible to human ACE2. It is unknown how easily the spike undergoes conformational change from ACE2 inaccessible to accessible. It is also unclear whether the accessible conformations with different RBD angles have a different binding affinity to ACE2. In addition, the residues of the spike that contribute to the high ACE2 binding strength need to be further analyzed based on the protein dynamics.

In an attempt to answer the above questions, the conformational distribution of the two spikes, the energy barriers between the ACE2-accessible and -inaccessible conformations, and the ACE2 accessibility and binding strength of the spikes at different RBD angles were explored with various computational methods in this Letter.

Based on 100 ns molecular dynamics (MD) simulations starting from two crystal structures (Figure S2, PDB IDs 2AJF26 and 6M0J27), the binding free energy (ΔG) of ACE2-RBD of the CoV-2-S is calculated to be −24.86 ± 0.59 kcal/
mol, which is much stronger than that of the CoV−S (−10.04 ± 0.66 kcal/mol) (Table 1) (see the Supporting Information).

| Table 1. Predicted Binding Free Energies (kcal/mol) of CoV-2-S RBD and CoV-5-S RBD to ACE2 Using the MM/GBSA Method |
|-----------------------------------|----------|----------|
| energy term | CoV-2-S RBD | CoV-5-S RBD |
| $E_{ele}$ | −86.91 ± 0.06 | −80.73 ± 0.07 |
| $E_{ele}$ | −697.07 ± 0.56 | −742.78 ± 0.71 |
| $E_{dp}$ | 760.94 ± 0.51 | 812.86 ± 0.67 |
| $E_{em}$ | −12.05 ± 0.06 | −10.34 ± 0.10 |
| $\Delta H$ | −35.10 ± 0.62 | −20.98 ± 0.64 |
| $-T\Delta S$ | −10.24 ± 0.56 | −10.94 ± 0.69 |
| $\Delta G$ | −24.86 ± 0.59 | −10.04 ± 0.66 |

The statistical error was estimated based on 50–100 ns MD simulation trajectory. A total of 1000 snapshots evenly extracted from the 50–100 ns MD trajectory of each complex was used for MM/GBSA calculations, with 10 snapshots for the entropy-term calculations.

Figure 1. Spike−ACE2 interaction spectra of CoV-2-S and CoV-5-S. The initial structures of MD simulations were modeled based on the 3D structures of 6ACG (A). The difference that is statistically significant at the 1% level (p < 0.01) was labeled with “+”. Key residues of CoV-2-S (B) and CoV-5-S (C) interacting with ACE2 were shown in sticks and colored green. Each residue contributed $\leq 1.00$ kcal/mol to the overall binding free energy.
interactions, CoV-2-S with larger RBD angles could form ACE2-inaccessible RBD angle (blue), ACE2-accessible RBD angle (green), and unavailable RBD angle (gray) of CoV-2-S trimer (B). Figure 2. Twenty aligned conformations extracted from the predicted conformational change pathway of CoV-2-S trimer between down and up states (A). The ACE2-inaccessible RBD angle (blue), ACE2-accessible RBD angle (green), and unavailable RBD angle (gray) of CoV-2-S trimer (B).

same as that for CoV-2-S (−18.00 ± 0.84 kcal/mol) but much stronger than that of the wild CoV–S (−10.59 ± 0.62 kcal/mol). This result demonstrated that the stronger ACE2 binding affinity of CoV-2-S could be quantitatively attributed to the 18 residue variations; the rest of the residue mutations of the spike outside the RBD–ACE2 interface have little effect on its ACE2 binding affinity.

The conformational change pathway of COV-2-S trimer between the down and up states was predicted by NUMD39 (Figures 2A and S5). Since the overall structure of RBD is insensitive to the RBD angle (Figure S6), we superimposed the RBD (6ACG) onto the 240 conformations extracted from the above predicted conformational transition pathway (Figure 2A) and found that there is no atomic collision between ACE2 and COV-2-S trimer if its RBD angle is greater than 52.2°. Accordingly, 52.2° should be regarded as the smallest accessible RBD angle of CoV-2-S to ACE2 (Figure 2B), which could be used as the criterion for classifying RBD up or down states. The results were also consistent with experimental structures; for example, the RBD angles of available RBD down conformations of CoV-2-S (Table S1) are less than 52.2°, and the smallest RBD up-state angle 54.8° in 6ACG is greater than 52.2°.

Based on the predicted conformational change pathway, we further investigated the correlation between the RBD angle and ACE2 binding affinity. As shown in Figure 3, the CoV-2-S has higher affinity with a larger RBD angle, with a correlation coefficient \( R^2 \) of 0.64. Despite having similar key interfacial interactions, CoV-2-S with larger RBD angles could form stronger hydrogen bonds with ACE2 by residues N487, Q498, and N501 and extra hydrogen bonds with K31 in ACE2 by residue Q493, as compared to smaller RBD angles (Figure S7). This result demonstrated that the virus could start to interact with host cells at an RBD angle of CoV-2-S as small as 52.2° and that the binding interaction with the host cell gets stronger and stronger as the RBD angle increases in size. When the RBD becomes almost fully opened (RBD angle = 84.6°), the binding free energy predicted based on 100 ns MD simulations could be as strong as −39.52 kcal/mol, which is much stronger than that of CoV-2-S with an RBD angle of 52.2° (−19.86 kcal/mol). A similar correlation between the RBD angle and ACE2 binding affinity can also be found for CoV–S with the \( R^2 \) of 0.58 (Figure S8).

Different from the stronger ACE2 binding strength of CoV-2-S RBD, bioassay results demonstrated a similar or lower binding affinity of the entire CoV-2-S than the entire CoV–S.17,18 We thought that CoV-2-S might have less ACE2-accessible conformations or be more difficult to change from down to up states. Thus, we performed two 4.8 μs vsREMD simulations30 to investigate the conformational dynamics of both the CoV-2-S and CoV–S trimers. We compared the predicted free energy surfaces (FESs) of 90 and 100 ns simulations and found that the simulation convergence was achieved after 90 ns (Figures S9 and S10). During the simulations, only D405 among the three RBD angle residues was found to undergo large fluctuations (Figure S11); therefore, we depicted the FES as a function of RBD angles and backbone RMSD (Figure 4). It revealed that CoV-2-S is more stable in down (ACE2-inaccessible) states. With the conformation accessibility in mind, we analyzed the accessible conformations and found that the population of one CoV-2-S RBD with an RBD angle ≥ 52.2° is 5.48%, while that of CoV–S is 22.7%, demonstrating that CoV-2-S has much fewer ACE2-accessible conformations than CoV–S. Recently, Lai et al. found that CoV-2-S has less structural flexibility than CoV–S according to their 350 ns MD simulations,31 and Ke et al. found that 53% pre-fusion structures of CoV-2-S trimer were closed with three down RBD using cryo-electron microscopy (cryo-EM),32 which further demonstrated that our result is in agreement with other computational and experimental results.

Noticeably, the free energy barrier from ACE2-inaccessible to -accessible states of CoV-2-S was calculated to be ~2.6 kcal/mol, which is bigger than that of CoV–S (~1.7 kcal/mol), indicating that CoV-2-S RBD is more difficult to become ACE2 accessible than CoV–S RBD (Figure 4C, D). More impressively, the free energy of CoV-2-S keeps going uphill as its RBD angle changes, until eventually reaching the fully up state. At least 4.4 kcal/mol is required for CoV-2-S to reach a
fully up state, while that for CoV–S is only 1.7 kcal/mol, which demonstrated that CoV–2–S is more difficult to change from down to fully up states. Therefore, the lower ACE2 binding affinity determined by using entire CoV–2–S might be, at least partially, attributed to less ACE2-accessible conformations and the more difficult conformational shift of CoV–2–S from ACE2 inaccessible to accessible.

CoV–2–S trimer can adopt two or three RBDs in up conformation, originating from either one-by-one or simultaneous RBD up-state motions. To study the possible coordinated movement among the three monomers, we found that while chain A is in RBD up state, chain C could be in RBD transitional states (down/up) from down to up (Figure 5A), suggesting that the up state of RBDs in the trimer

Figure 4. Conformational distribution of CoV–2–S and CoV–S trimers predicted by vsREMD simulations. The free energy surface of CoV–2–S (A) and CoV–S (B) trimers as a function of RMSD against initial structures and RBD angles of chain A, together with minimum energy pathway between down and up states. The free energy along the conformational change pathway from down to up states for CoV–2–S (C) and CoV–S (D) trimers.

Figure 5. Coordinated motions among the three chains of CoV–2–S trimer. (A) A conformation of CoV–2–S trimer with one RBD in the up state (chain A, colored pink) while another RBD is in down/up transition (chain C, colored blue), compared with three down RBD conformations (colored gray). The correlation between the RBD angles (deg) in chain A and chain B or chain C during the vsREMD simulations (B).
NUMD was analyzed by D3Pockets. Five pockets were conformational change pathway of CoV-2-S predicted by additional druggable sites to keep CoV-2-S closed, the important in conformational dynamics. To evaluate great potential to keep CoV-2-S down (Figure S12D). (S324A, F322A, R321A, N329A, and T167B) also shows its hydrogen bonds with RBD and NTD in adjacent chains ligand binding should be able to keep RBD down. After a the prevention of Pocket 2 or Pocket 4 from getting larger by both Pockets 2 and 4 become larger (Figure 6C). Therefore, RBD

**Figure 6.** Predicted potential ligand binding sites of CoV-2-S. Five potential ligand-binding pockets (A). The redder the pocket grids are, the more stable the subpocket regions throughout the pathway are. Pocket correlation between the five predicted binding pockets (B). The numbers are the correlation coefficients between two pockets during conformational changes from down to up states. The pocket correlation between Pocket 2 and Pocket 4 (C).

might be one by one. When chain A is in RBD down/up transition, chain C has larger RBD angles than chain B (Figure 5B), indicating that chain C tends to be more RBD up when chain A is up. The population of chain C at down/up transitions (40° < RBD angle < 52.2°) is 4.6%, which is 7.2% for that of CoV–S. By the structural analysis of chain A, we found that the NTD (N-terminal domain) in chain A lays down ~2.4 Å when its RBD is up, indicating that the conformational changes of the NTD in chain A resulted in the bigger tendency of chain C to get up.

One strategy to block the ACE2 binding is to freeze CoV-2-S at its ACE2-inaccessible state. For example, linoleic acid has been reported to be able to compact the RBD trimer by interacting with two adjacent RBDs together. To find additional druggable sites to keep CoV-2-S closed, the conformational change pathway of CoV-2-S predicted by NUMD was analyzed by D3Pockets. Five pockets were identified that are dynamically correlated with RBD’s up movement (Figures 6A, B). For instance, Pocket 2 has a positive volume correlation ($R^2 = 0.88$) with Pocket 4 along RBD’s up movement. In detail, as RBD becomes more open, both Pockets 2 and 4 become larger (Figure 6C). Therefore, the prevention of Pocket 2 or Pocket 4 from getting larger by ligand binding should be able to keep RBD down. After a literature survey, eight compounds were found as the spike’s blockers, but their binding sites on the spike are unknown (Table S4). We docked them into the five pockets and found that the eight compounds have great possibility of being bound in the five pockets among all the 39 druggable pockets predicted by D3Pockets. In particular, quercetin binds to Pocket 5 at the RBD down state of CoV-2-S with the strongest binding strength of −9.04 kcal/mol among all the 39 pockets (Figures S12A–C). The binding mode of quercetin that forms hydrogen bonds with RBD and NTD in adjacent chains (S324A, F322A, R321A, N329A, and T167B) also shows its great potential to keep CoV-2-S down (Figure S12D).

Noticeably, the glycosylation of CoV-2-S was reported to be important in conformational dynamics. To evaluate whether the glycosylation significantly affects its ACE2 binding affinity and its movement from RBD down to RBD up, we constructed glycosylated CoV-2-S and CoV–S according to experimental data (Tables S5 and S6). Obviously, the five predicted pockets are not shielded by the added glycans (Figure S13A). For a small RBD angle, the ACE2 binding affinity of the glycosylated CoV-2-S was calculated to be $-19.14 \pm 0.72$ kcal/mol (Table S7), which is similar to that of CoV-2-S without glycosylation ($-18.00 \pm 0.84$ kcal/mol). Similarly, the glycosylation of CoV-2-S with a large RBD angle also does not obviously impact its ACE2 binding affinity. Next, we performed adaptive steered molecular dynamics (ASMD) simulations of the glycosylated CoV-2-S and CoV–S along the RBD angles (see the Supporting Information for details). The potential mean of force (PMF) of the glycosylated RBD-down CoV-2-S (16.9 kcal/mol, Figure S13B) to RBD up is obviously larger than that of the glycosylated CoV–S (12.0 kcal/mol), which further confirms the results of the vsREMD simulations.

In conclusion, our results showed that 52.2° could be the criterion to classify ACE2-accessible and -inaccessible conformations. Among the accessible conformations, the ACE2 binding strength becomes stronger and stronger as the RBD angle increases. The ACE2 binding strength of CoV-2-S is always higher than that of CoV–S at different RBD angles. However, CoV-2-S has less ACE2-accessible conformations than CoV–S, and the ACE2-inaccessible conformations of CoV-2-S are more difficult to undergo conformational shift to the accessible state than CoV–S. Therefore, the observed similar or lower ACE2 binding affinity of entire CoV-2-S could be attributed to less ACE2-accessible conformations and the more difficult transition from ACE2-inaccessible to -accessible conformations in solution. On the basis of the structural dynamics simulated, key residues of CoV-2-S for the strong binding strength to ACE2 were analyzed and five pockets were proposed as potential binding sites for drug discovery and development against CoV-2-S.
**ASSOCIATED CONTENT**

† Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jpclett.0c02958.

Details of the methods used and the figures and tables discussed in this study (PDF)

Supporting files, including Jmol files and five suggested pockets (ZIP)

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Author Contributions

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Notes

The authors declare no competing financial interest.

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