Study of Specific Receptor Binding Mode Suggests a Possible Enzymatic Disinfectant for SARS-CoV-2

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ABSTRACT: The outbreak of the coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2 has spread globally. SARS-CoV-2 enters human cells by utilizing the receptor-binding domain (RBD) of an envelope homotrimeric spike (S) glycoprotein to interact with the cellular receptor angiotensin-converting enzyme 2 (ACE2). We thoroughly studied the differences between the two RBDs of SARS-CoV and SARS-CoV-2 when they bind with ACE2 through molecular dynamics simulations. The peculiarities of the SARS-CoV-2 RBD are obvious in several aspects such as fluctuation of the binding interface, distribution of binding free energy on residues of the receptor-binding motifs, and the dissociation process. Based on these peculiarities of SARS-CoV-2 revealed by simulations, we proposed a strategy of destroying the RBD of SARS-CoV-2 by employing enzymatic digestion. This unique strategy is promising for developing a skin-friendly, nontoxic, and convenient disinfectant to protect people from infection by SARS-CoV-2.

INTRODUCTION

The emergence of the highly pathogenic coronavirus SARS-CoV-2 poses a serious global health emergency, causing a devastating societal and economic burden. The virus has spread worldwide. Similar to individuals infected by SARS-CoV in 2003, patients with COVID-19 showed symptoms of fever, severe respiratory illness, and pneumonia. SARS-CoV-2 entry into human cells is mediated by an envelope homotrimeric spike (S) glycoprotein protruding from the viral surface. There are two subunits in S glycoprotein: one is responsible for binding to the host cell receptor angiotensin-converting enzyme 2 (ACE2) and the other promotes fusion of the viral and cellular membranes. The S glycoprotein of SARS-CoV-2 shares 80% amino acid sequence identity with SARS-CoV. A recent high-resolution structure of the SARS-CoV-2 receptor-binding domain (RBD) bound to ACE2 has shown that the ACE2-binding mechanism is apparently identical between SARS-CoV-2 and SARS-CoV RBDs.

Although SARS-CoV-2 shares many similarities with SARS-CoV, it is more likely to spread from human to human. The origin of its higher infectivity remains puzzled. McLellan et al. previously observed that ACE2 can bind to the SARS-CoV-2 S ectodomain with ~15 nM affinity which is approximately 10- to 20-fold higher affinity than ACE2 binding to SARS-CoV S. This high affinity of SARS-CoV-2 S for ACE2 was considered to contribute to the apparent ease of spread between humans. To the contrary, some experiments obtained similar binding affinities for ACE2 binding to RBDs of two viruses. Different simulations also showed distinct results, probably because of different free energy calculation methods, different simulation settings (temperature and force field), and different complex structures employed. Beyond this, experiments showed that the SARS-CoV-2 RBD does not have antibody cross-reactivity with RBDs of SARS-CoV and MERS-CoV. This indicated that anti-RBD antibodies are viral species-specific inhibitors and inherent differences between coronavirus exist. Therefore, thoroughly understanding the differences between two RBDs of SARS-CoV and SARS-CoV-2 can provide a structural basis for the rational design of vaccine and disinfectant. Previous studies mainly focused on simulation of the binding structure of the complex, comparison of the binding affinity with ACE2 between two RBDs, and analysis of hot spots of the binding interface among others. Our simulations aimed to elucidate the different interface dynamics and binding interaction distributions of two RBDs and its influence on the dissociation process of complexes. Some information of the mechanism about the recognition of two proteins was also shown.

As the available treatment or vaccine has not obtained a complete success, this virus might be with us for a long time and the ability to protect people from infection by SARS-CoV-2 is of great concern. Many hard surface disinfectants have been proved to be effective against SARS-CoV-2 but are not suitable for use on skin or personal protective equipment.
For example, PPE should be effectively disinfected to extend the use and reuse due to shortages of N95 masks and other protective PPE. A previous study suggested that ethanol and chlorine-based disinfectants are not suitable for decontamination because of their capacity to adsorb on the fabric material and interfere with their electrostatic properties, thereby reducing the filtration capacity. Some recommended PPE decontamination methods (UV, dry heat, and hydrogen peroxide vapors) cannot, however, be easily applied for daily use of PPE, warranting the need for new or additional disinfection methods that can be applied in times of need in these settings without causing harm to adjacent skin. In addition to this, the use of nasal sprays to minimize person-to-person transmission of the virus is also needed, because of the dispersal of the SARS-CoV-2 virus from asymptomatic individuals carrying high viral loads in their nasal epithelium. Therefore, a skin-friendly, nontoxic, and convenient disinfectant used for decontamination of PPE or nasal sprays is urgently needed.

Based on thorough study of the binding mode between SARS-CoV-2 and ACE2, we proposed a strategy that can principally destroy the SARS-CoV-2 RBD by employing endopeptidase. This enzymatic strategy has a great potential for the disinfection of skin and PPE and for the development of nasal sprays.

RESULTS AND DISCUSSION

Different Interface Dynamics and Binding Interaction Distributions. The RBDs of SARS-CoV and SARS-CoV-2 can be structurally divided into two parts: a core region and a receptor-binding motif (RBM). The core region which consists of five antiparallel strands of β-sheet shares 87% sequence identity between the two viruses and is highly conserved. In contrast, there are more mutations in the RBM, only sharing 50% homology. As shown in Figure 1a,b, there are three binding contact regions in the RBM named R1, R2, and R3. These three regions bind with different sites of ACE2 and anchor the RBD firmly with ACE2. The homology between the two RBMs in R1, R2, and R3 is 30, 70, and 55%, respectively. The sequence variations mainly exist in R1 and R3, two loop regions.

Mutations in the RBM might bring obvious differences between the two RBDs of SARS-CoV and SARS-CoV-2 when they bind with ACE2. To understand the two modes of binding at the atomic level, we analyzed 10 μs-long trajectories of the complexes of SARS-CoV S and SARS-CoV-2 S bound to ACE2 provided by Shaw group. The overall root mean square deviation (RMSD) of both the complexes approximately reached an equilibrium during the last 2-μs-long simulations (Figure S1). However, the RMSD of the RBM of the two RBDs (Figure 1d) reveals different interface dynamics. The RBM of the SARS-CoV-2 RBD is more active than that of SARS-CoV.
the SARS-CoV RBD, which demonstrates that the SARS-CoV-2 RBD in the complex is more flexible and can change conformation easily with lower energy barrier. The per-residue root mean square fluctuations (RMSF) of ACE2 in the two complexes did not show obvious difference (Figure 1c), while more fluctuation can be observed in RMSF of the SARS-CoV-2 RBD than that of the SARS-CoV RBD (Figure 1f). Residues from Ser443 to Asn450 in the SARS-CoV-2 RBD are especially more fluctuant than the corresponding residues in the SARS-CoV RBD, which contributes to the higher flexibility of the SARS-CoV-2 RBD (Figure 1c, regions masked by black circles). Most of these residues are located in the R3 of the RBM, and therefore, we can also observe that the RMSD of the R3 in the SARS-CoV-2 RBM is more fluctuant than SARS-CoV (Figure S2). This predicts that the binding affinity of R3 may be weakened in SARS-CoV-2 bound to ACE2. This was verified in the latter analysis of binding free energy. The origin of the above difference between the two RBMs in this region is attributed to the four mutations (Ser443, Lys444, Val445, Gly446) of the SARS-CoV-2 RBM (Figures 1a and S3).

The above results indicate that different interaction patterns at the binding interface are adopted for these two complexes. We calculated the binding free energy of the two complexes and decomposed the binding energy into each pair of residues at the interface. The SARS-CoV-2 binds to ACE2 stronger than SARS-CoV by 5.6 kcal/mol due to enhanced electrostatic and van der Waals interactions (Table 1). Figure 2a shows the distribution of these residues on the RBM of SARS-CoV and SARS-CoV-2. The higher binding affinity of SARS-CoV-2 RBD made it difficult to be dissociated and start dissociating after longer simulation. SARS-CoV&ACE2 and SARS-CoV-2&ACE2 started dissociating at 1.3 ns (red dotted line) and 3.5 ns (blue dotted line), respectively. We found that the dissociation of SARS-CoV-2&ACE2 is obviously controlled by the Coulomb interaction. The long-range interaction from Coulomb distribution could make SARS-CoV-2 RBD easier to recognize ACE2 compared with the SARS-CoV RBD. The distances of Cz of 10 residue pairs obtained in Figure 2 during SARS-CoV-2&ACE2 dissociation show the coordinated distance variation of first 8 pairs and the changes are smaller than SARS-CoV&ACE2 (Figure 3c,d,e). Interestingly, the two residue pairs maintain approximately constant distance during the dissociation in both cases, which are Y-N (Tyr83&Asn473) and Q-N (Gln24&Asn473) in SARS-CoV&ACE2 and Y-N (Tyr83&Asn487) and Q-A (Gln24&Ala475) in SARS-CoV-2&ACE2, respectively (Figures 3d,e and S4). The residues Tyr83 and Gln24 lie near the N-terminal of the helical bundle of ACE2. The residue Asn473 of the SARS-CoV RBD and the residue Asn487, Ala475 of SARS-CoV-2 RBD are located at the R1 region of the RBM (Figures S4 and 3h,k). Therefore, we could assume that the N-terminal of the helical bundle of ACE2 might be first recognized by R1 of the RBD in the binding dynamics and plays an important role in the whole binding process. These results also show that SARS-CoV and SARS-CoV-2 RBDs is 5.6 kcal/mol.

Table 1. Binding Free Energy $\Delta G_{bind}$ of SARS-CoV-2&ACE2 and SARS-CoV&ACE2 Complexes Using MM/GBSA Method

| Method | SARS-CoV-2 & ACE2 | SARS-CoV & ACE2 |
|--------|------------------|------------------|
| van der Waals | $-88.3 \pm 5.0$ | $-75.7 \pm 4.9$ |
| electrostatic | $-724.3 \pm 24.6$ | $-683.9 \pm 21.9$ |
| $E_{vdW}$ | $790.9 \pm 23.6$ | $740.0 \pm 19.9$ |
| $E_{int}$ | $-13.4 \pm 0.6$ | $-9.9 \pm 0.6$ |
| $\Delta G_{bind}$ MM-GBSA | $-35.1 \pm 5.4$ | $-29.5 \pm 5.6$ |

**Different Dissociation Process.** We constructed an “artificial” dissociation model to gather some information on the recognition mechanism in the binding process of ACE2 and the two RBDs. The mass centers of ACE2 and RBD separated along the x direction at a constant speed, as shown in Figure 3a. During the dissociation, the force applied on SARS-CoV-2&ACE2 lasts longer than that applied on SARS-CoV&ACE2 (Figure 3b). This demonstrates that more effort is needed to dissociate the complex of the SARS-CoV-2 RBD and ACE2. This can also be verified in Figure 3c, which shows the interaction energy between ACE2 and the two RBDs. The higher binding affinity of the SARS-CoV-2 RBD makes it difficult to be dissociated and start dissociating after longer simulation. SARS-CoV&ACE2 and SARS-CoV-2&ACE2 started dissociating at 1.3 ns (red dotted line) and 3.5 ns (blue dotted line), respectively. We found that the dissociation of SARS-CoV-2&ACE2 is obviously controlled by the Coulomb interaction. The long-range interaction from Coulomb distribution could make SARS-CoV-2 RBD easier to recognize ACE2 compared with the SARS-CoV RBD. The distances of Cz of 10 residue pairs obtained in Figure 2 during SARS-CoV-2&ACE2 dissociation show the coordinated distance variation of first 8 pairs and the changes are smaller than SARS-CoV&ACE2 (Figure 3d,e). Interestingly, the two residue pairs maintain approximately constant distance during the dissociation in both cases, which are Y-N (Tyr83&Asn473) and Q-N (Gln24&Asn473) in SARS-CoV&ACE2 and Y-N (Tyr83&Asn487) and Q-A (Gln24&Ala475) in SARS-CoV-2&ACE2, respectively (Figures 3d,e and S4). The residues Tyr83 and Gln24 lie near the N-terminal of the helical bundle of ACE2. The residue Asn473 of the SARS-CoV RBD and the residue Asn487, Ala475 of SARS-CoV-2 RBD are located at the R1 region of the RBM (Figures S4 and 3h,k). Therefore, we could assume that the N-terminal of the helical bundle of ACE2 might be first recognized by R1 of the RBD in the binding dynamics and plays an important role in the whole binding process. These results also show that SARS-CoV and
SARS-CoV-2 adopt similar recognition mechanisms to bind with ACE2.

**Design of a New Disinfectant.** Based on the understanding of the binding mode of SARS-CoV-2 RBD and ACE2, we proposed a strategy to destroy the SARS-CoV-2 RBD by employing endopeptidase. Enzyme preparation for disinfection is a safe, skin-friendly, convenient way to block the spread of SARS-CoV-2. Endoproteinases are proteolytic peptidases that can break the peptide bonds of nonterminal residues. They are usually very specific for certain residues and can selectively cleave certain positions of a protein. For example, glutamyl endopeptidase can hydrolyze peptide bonds after the negatively charged Glu or Asp residues, with a higher preference to the former. We used five kinds of endopeptidases, which have preferential cleavage of Glu, Gly, Lys, Pro, or Ala, to cut the SARS-CoV-2 RBD (Figure 4). There are 5, 14, 8, 9, and 10 cleavage sites on the RBD of Glu, Gly, Lys, Pro, and Ala, respectively. Therefore, the SARS-CoV-2 RBD will be cut into several fragments by specific endopeptidases. Results of molecular dynamics simulation showed that the conformation of the RBM of the cleaved RBD has an obvious change except for the case of Ala cleavage. For the cases of Glu, Gly, and Pro cleavage, the R1 of the RBM, which plays an important role in ACE2 recognition, is almost completely destroyed. Hence, Glu, Gly, and Pro cleavage can result in the failure of its binding with ACE2. Although the R1 of the RBM in the case of Pro is mainly preserved after cleavage, it bends a lot with a smaller angle between R1 and R2, also inhibiting its binding with ACE2 (Figure S6). The Ala cleavage brings a tiny change of the RBM conformation and its binding with ACE2 cannot be blocked (Figures 4 and S7). Our simulations indicated that endopeptidases which can selectively cleave Glu, Gly, Lys, and Pro are able to destroy the structure of the RBM of SARS-CoV-2. These endopeptidases can be utilized as the major components of new disinfectants to block the virus spread.

**CONCLUSIONS**

We thoroughly studied the different characteristics of SARS-CoV-2 compared with SARS-CoV using molecular dynamics simulations. A different binding mode is adopted in the complex of SARS-CoV-2&ACE2. The dissociation process of the binding complexes reveals that R1 of the RBD is vital in ACE2 recognition. The conformation destruction of R1 might lead to the failure of binding with ACE2. These findings inspire us to propose a endopeptidase-based disinfectant for the disinfection of SARS-CoV-2, which is suitable for application on skin, PPE, and in nasal sprays. The peculiarities of SARS-CoV-2 revealed by our simulations and the proposed strategy of designing the disinfectant provide some guidance for the development of clinical interventions in the future.

**METHODS**

**MD Simulation.** All molecular dynamics simulations were performed using GROMACS 2019.3. The simulations used the Amber ff99SB-ILDN force field for proteins and the TIP3P model for water. The initial structures of SARS-CoV and SARS-CoV-2 bound to ACE2 were from equilibrium structures in 10 μs-long trajectories.
The simulations from Shaw group were initiated from ACE2 in complex with the RBD of S protein from SARS-CoV (PDB ID: 2AJF) and SARS-CoV-2 (PDB ID 6M17). In order to mimic physiological conditions, the system was neutralized and salted with NaCl, with a final concentration of 0.15 M. The default setting of protonation states of the charged residues in GROMACS was adopted and adjusted on the basis of local hydrogen-bonding interactions, salt bridge, and Zinc finger. The protein was placed in a periodic cubic box of water molecules represented by the three-point charge TIP3P model, whose boundary is at least 15 Å.

Figure 3. Dynamical dissociation of the two complexes. (a) Dissociation process: the complexes were placed in a rectangular box and the line connecting the two mass centers was parallel to the x-axis. The mass centers of ACE2 and the RBD were pulled in opposite directions along the x-axis at a constant speed. (b) Force applied on the system in the dissociation process. (c) Interaction energy between ACE2 and the two RBDS, Coulomb distribution, and van der Waals distribution during dissociation. (d) Distances of Cα of 10 residue pairs obtained in Figure 2 during SARS-CoV&ACE2 dissociation. Line color from deep red to dark blue is corresponding to the decreased binding energy of the pairs. (e) Distances of Cα of 10 residue pairs obtained in Figure 2 during SARS-CoV-2&ACE2 dissociation. Line color from deep red to dark blue is corresponding to the decreased binding energy of the pairs. (f–h) Complex structures of SARS-CoV&ACE2 at 1.3, 8, and 12 ns, respectively. (i–k) Complex structures of SARS-CoV-2&ACE2 at 3.5, 8, and 12 ns, respectively.

Figure 4. Design of a new disinfectant. Endopeptidases which can selectively cleave Glu, Gly, Lys, Pro, and Ala were employed to destroy the SARS-CoV-2 RBD. The SARS-CoV-2 RBD without cleavage is in blue color and the RBD cleaved by endopeptidases is in red color.

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from any protein atoms. The total system was energy minimized by a succession of steepest descent and conjugate gradient methods. Thereafter, the solvent was equilibrated for 10 ns at constant temperature (310 K) and pressure (1 bar) (NPT) by restraining the positions of the protein atoms followed by NPT equilibration for another 100 ns without position restrain. The final production runs (NPT) lasted for 100 ns. The binding free energies (∆G_binding) were calculated with the Molecular Mechanics Generalized Born Surface Area (MM-GBSA) method using MMPBSA.py^32 on 200 frames taken from the final production runs at constant temperature (310 K) and pressure (1 bar) (NPT). In our calculations, the entropic contribution of the free energy was not considered. We used V-rescale thermostat^33 and Parrinello–Rahman barostat^34 to keep the temperature and pressure constant, respectively. The cutoff radius for neighbor searching and nonbonded interactions was taken to be 10 Å, and all bonds were constrained using the LINCS algorithm. All the simulation results were visualized by VMD.36

Disinfectant Design. Five kinds of endopeptidases, which have preferential cleavage of Glu, Gly, Lys, Pro, or Ala, were cut to the SARS-CoV-2 RBD, were selected as candidates for disinfectant design. The peptide bond of the corresponding cleavage sites on the RBD of Glu, Gly, Lys, Pro, and Ala was broken in MD simulation settings. The cleaved structure was equilibrated for 200 ns of binding free energies (∆G_binding) were calculated with the Molecular Mechanics Generalized Born Surface Area (MM-GBSA) method using MMPBSA.py^32 on 200 frames taken from the final production runs at constant temperature (310 K) and pressure (1 bar) (NPT). In our calculations, the entropic contribution of the free energy was not considered. We used V-rescale thermostat^33 and Parrinello–Rahman barostat^34 to keep the temperature and pressure constant, respectively. The cutoff radius for neighbor searching and nonbonded interactions was taken to be 10 Å, and all bonds were constrained using the LINCS algorithm. All the simulation results were visualized by VMD.36

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.langmuir.0c02911.

Graphical representations of RMSD of two complexes versus simulation time; residues in SARS-CoV-2 RBD different from SARS-CoV RBD; water molecules accessible to the binding interface of SARS-CoV&AACE2 and SARS-CoV-2&AACE2; residue pairs with approximately constant distance during dissociation; and structural superimposition of SARS-CoV-2 RBD and cleaved RBD by glycol endopeptidase (PDF)

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Author Contributions
Y.C. and J.G. conceived the idea. Y.C. performed the simulation and analyzed the data. J.G. supervised the project. Y.C., J.G. wrote the paper.

Notes
The authors declare no competing financial interest.

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