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Quantitative *in silico* analysis of SARS-CoV-2 S-RBD omicron mutant transmissibility

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ARTICLE INFO

Keywords:

SARS-CoV-2 omicron valiant
ACE-2
Binding affinity
Quantitative *in silico* analysis
Molecular interaction energy
Docking inhibitor

ABSTRACT

Covid-19 variants transmissibility was quantitatively analyzed *in silico* to understand the reaction mechanisms and to find the reaction inhibitors. Especially, SARS-CoV-2 omicron mutant (omicron S-RBD) binding affinity with human angiotensin-converting enzyme-2 (ACE-2) was quantitatively analyzed using molecular interaction (MI) energy values (kcal mol^{-1}) between the S-RBD and ACE-2. The MI of their optimized complex structures demonstrated that *omicron*'s MI value (749.8) was 1.4 times delta MI (538.1) and 2.7 times alpha MI (276.9). The omicron S-RBD demonstrated the most vital transmissible strength. The 14 currently proposed medical treatment compounds did not show as the inhibitors to block the omicron S-RBD and ACE-2 binding; instead, they adsorbed at the ACE-2 active site and may inhibit the ACE-2 activity. A modified candidate (Gallo catechin gallate) whose two phenolic hydroxy groups were replaced with two carboxy groups was repulsed from ACE-2, indicating that further modification of medical treatment candidates may produce an effective docking inhibitor.

1. Introduction

Variants of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have spread rapidly throughout the entire world [1,2]. The docking of SARS-CoV-2 with Angiotensin-converting enzyme 2 (ACE-2) causes more severe infection than influenza [3]. The stereo structure of the SARS-CoV-2 and ACE-2 complex was determined, and the contact site of SARS-CoV-2 with ACE-2 was clarified [4]. The transmissible strength could be chemically related to the feasibility of the binding between spiked receptor-binding domain (S-RBD) and ACE-2. The theoretical proposal for explaining the contact strength was quantitatively described using the reproducible chromatographic retention time, including enantiomer recognition and protein affinity [5]. The fundamental technical method is explained in detail in the online textbook "Quantitative *in silico* analytical chemistry". The strongest molecular interaction (MI) is the ion-ion (strong electrostatic (ES)) interaction, and the tightness follows hydrogen-bonding (HB), and the van der Waals (VW) force is the weakest interaction [6]. The approach was applied for quantitative analysis of enzyme reactivity, such as alanine racemase, serine racemase, alcohol dehydrogenase, cinnamyl alcohol dehydrogenase, D-amino acid oxidase (DAO), and D-aspartic acid oxidase (DDO) [7]. Furthermore, the newly developed

analytical method was applied for quantitative *in silico* analysis of SARS-CoV-2 S-RBD mutant's binding affinity with ACE-2 [8]. The transmissible strength of Covid-19 variants can be analyzed based on the complex structure of SARS-CoV-2 mutants and ACE-2. The downloaded stereo structure of SARS-CoV-2 with the ACE-2 complex (RSC PDB:7mjk) indicates that several acidic amino acids exist at the ACE-2 contact site [9].

First, the MI energy values between amino acids of S-RBD and ACE-2 were calculated. A selected amino acid from ACE-2 was glutamic acid, and several amino acids from the contact site of S-RBD were arginine, asparagine, aspartic acid, glutamine, leucine, lysine, threonine, and tyrosine. The initial study indicated that the delta mutant having two mutated basic amino acids (L452R, T478K) demonstrated the high MI energy values due to the strong electrostatic interaction between the S-RBD arginine and lysine and ACE-2 aspartic acid.

In further *in silico* analysis, docking was performed between ten S-RBD mutants with two contact site peptides of ACE-2 (S19-S105 and E329-N394). The four end amino acids of the selected ACE-2 peptides were locked to maintain their stereo structure. This modification was applied based on the calculation capability of a desktop computer. The theoretically calculated MI (binding) energy values of final (optimized) structure (FS) (MIFS) energy values of S-RBD mutants and ACE-2 are

Abbreviations: SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; S-RBD, Spiked receptor-binding domain; ACE-2, Angiotensin-converting enzyme-2; MI, Molecular interaction; ES, Electrostatic; HB, Hydrogen bonding; VW, van der Waals; FS, Final (optimized) structure.

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<https://doi.org/10.1016/j.talanta.2022.123206>

Received 27 December 2021; Received in revised form 31 December 2021; Accepted 3 January 2022

Available online 7 January 2022

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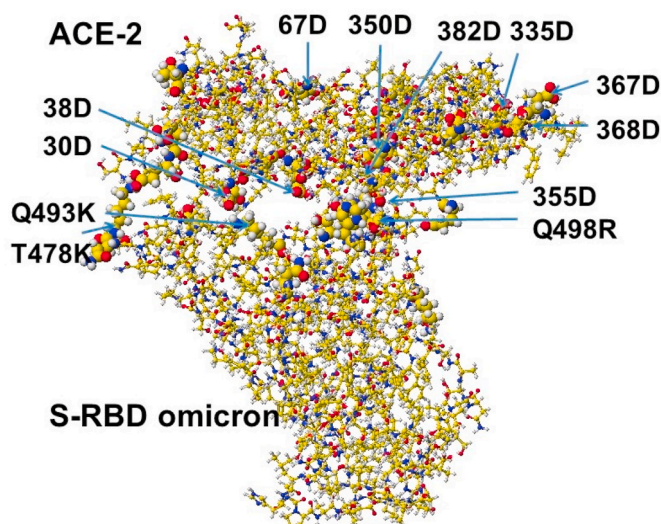


Fig. 1. Structure of omicron S-RBD and a part of ACE-2 complex.

followed; $\alpha < \beta = \gamma < WT < \mu, \kappa(B.1.621) < \delta\text{-plus} < \kappa(B.1.617.1) < \lambda < \theta < \delta$; $276.9 < 289.2 < 300.1 < 301.9 < 406.8 < 414.1 < 503.5 < 509.6 < 538.1 \text{ kcal mol}^{-1}$ [8]. The S-RBD *delta* mutant demonstrated high MI energy values with the extracted ACE-2 peptides. The MI energy value of *delta* mutant is 1.9 times that of *alpha* mutant. The result supported the large spread of *delta* mutant. Their calculated MI energy difference supported the weaker transmissibility of the *delta*-plus mutant compared to that of the *delta* mutant. Because K417 N mutation, replacing the ion-ion interaction to hydrogen bonding, reduces MI energy value.

According to News, the multi-mutated *omicron* Covid-19 variant has been alert for transmissibility and toxicity [10]; therefore, further analysis was performed to study the transmissibility of the *omicron* mutant. The list of mutated amino acids was obtained from Ref. [11]. The calculated MI energy values are used to evaluate the transmissibility strength. The infection is caused by contacting the S-RBD with ACE-2, and the contact-blocking compounds are candidate inhibitors. Previously, 54 compounds collected among flu medicine ingredients, acidic drugs, candidate treatment medicines for SARS-CoV-2 patients from News, and acidic compounds were studied to analyze the binding affinity with S-RBD mutants and ACE-2. The acidic compounds repulsed from ACE-2 were selected for further analysis; however, the basic compounds were eliminated because they were tightly contacted with ACE-2 and should hinder ACE-2 enzyme activity. In addition, the feasibility of the proposed 14 treat-medicine candidates was studied as the blocking inhibitors.

2. Experimental

The manual docking process was the same as that used for quantitative analysis of molecular recognition degree in chromatographically and quantitative analysis of enzyme reactivity. The basic complex structure of SARS-CoV-2 and ACE-2 was from Ref. [4]. The amino acids of the extracted S-RBD were mutated based on the reference [2], and the mutated structures were optimized using the MM2 program. Then, the mutated S-RBD was superimposed on the original reference structure; then, the new S-RBD and ACE-2 formed a complex in the optimization process. The MI energy values were obtained from their original and the complex's values using the following equation: $MIFS = \{fs(S\text{-RBD}) + fs(ACE\text{-}2)\} - fs(S\text{-RBD and ACE-}2 \text{ complex})$, where fs is the final structure energy value of individual molecule, and FS is the final structure energy value of the complex. HB, ES, and VW indicate final (optimized) structure, hydrogen bonding, electrostatic interaction, and van der Waals force.

Computational chemical calculations were performed using a DCPIx86-based PC with an Intel Core™i7-2600 cpu 3.40 GHz (Dospara, Yokohama) with the CAChe program (Fujitsu, Tokyo). The minimum energy level was $10^{-7} \text{ kcal mol}^{-1}$.

3. Results and discussion

The new variant omicron S-RBD contains basic amino acid lysine mutated from asparagine (N440K), threonine (T478K), and glutamine (Q493K), arginine mutated from asparagine (Q498R), and other mutated amino acids contributed hydrogen bonding (G446S, G496S). The omicron mutant S-RBD should tightly contact ACE-2 compared to *delta*-mutant, containing one mutated lysine (T478K) and one mutated arginine (L452R). The *omicron*-mutated S-RBD was docked with ACE-2 and calculated the molecular interaction (MI) energy value, as previously calculated for various mutants. The calculated MI energy value of optimized final structure energy (MIFS) was very high ($749.8 \text{ kcal mol}^{-1}$) compared with those other mutants [8]. Fig. 1 shows the complex conformation.

Key amino acid residues are indicated as 0.5 atomic sizes, and other amino acids are done as 0.2 atomic sizes.

The location of ACE-2 glutamic acid is indicated. Another acidic amino acid is aspartic. Black, dark gray, light gray, and white balls are oxygen, nitrogen, carbon, and hydrogen.

Critical amino acid residues are indicated 0.5 atomic sizes, and other amino acids are shown 0.2 atomic sizes in Fig. 1. The carboxy group oxygen atoms of ACE-2 are indicated as 0.5 atomic size black ball. The location of aspartic acid (D) of ACE-2 is indicated, and other acidic amino acids are glutamic acid (E). The location of several key mutated amino acids of S-RBD is also indicated, and these basic amino acids are contacted with ACE-2 acidic amino acids. The conversion from glutamine to arginine or lysine enhanced the binding affinity with ACE-2. The mutation from threonine to lysine was also increased the tight adsorption due to ion-ion interaction. High MIES values supported these phenomena.

The primary interaction force contributing to the binding affinity between omicron S-RBD and ACE-2 was electrostatic (ES) interaction, and the MIES energy value was $549.1 \text{ kcal mol}^{-1}$, followed by hydrogen bonding (HB) ($287.8 \text{ kcal mol}^{-1}$). Especially, the mutation of T478K of *delta*-S-RBD demonstrated a strong contact with ACE-2 [8]. However, the omicron mutant contains additional lysins, N440K and T478K; those tightly bound with the contact site acidic amino acids of ACE-2.

Increasing binding strength was also found in other mutants. The mutation E484K was found in *beta*, *gamma*, *kappa* (B.1.621), *mu*, and *theta* mutants. This mutation from glutamic acid (E) to lysine (K) enhances the ion-ion interaction with ACE-2. The mutation of L452R from neutral leucine (L) to basic arginine (R) also strengthened the binding. The initial experiment using calculated MI energy values between one acidic amino acid (glutamic acid of ACE-2) and one amino acid of S-RBD supported the binding strength. On the other hand, K417 N mutation reduced the binding strength.

The steric hindrance and the number of basic amino acids affect the binding affinity. In the omicron virus multiplication process, supplying excess basic amino acids is required. The "Healthy eating" report presented the regional food habitude [12], suggesting transmissibility and mortality are very high in certain countries. Excess eating dairy and animal protein seems to relate to the urgent problem.

Previously, 54 treatment medicine candidates were investigated as potential docking inhibitors of ACE-2 contact site with S-RBD, including ingredients in flu medicines, foods, and proposed compounds in several News. The effective inhibitors were m-carboxyl-L-tyrosine, citric acid and citric acid glycosides, ferulic acid, gallic acid, glycyrrhizic acid, ibuprofen, lactic acid, malic acid, mefenamic acid, nalidixic acid, and naproxen. Only acidic compounds were repulsed from ACE-2; however, the carboxyl group of aspirin did not inhibit the contact, rather bridged the connection of S-RBD lysine and ACE-2 arginine. The molecular size

Table 1
Molecular interaction values between an analyte and ACE-2.

Compound	MIFS	MIHB	MIES	MIVW
Baricitivir	130.8166	160.670	-13.210	-15.380
Carcitriol	122.4180	144.948	-16.333	-9.458
Dexamethasone	169.3355	178.940	44.341	-15.208
Ivermectin B1b	164.4401	169.012	-7.959	5.777
Losartan	148.8950	166.159	-1.967	1.794
N-Chloroacetyl-N'-sulfonamido-piperazine	140.3778	168.391	-11.246	-16.554
N-Chloroacetyl-pipedinyl-4-carboxamide	153.2242	177.111	-1.543	-21.668
Gallocatechin gallate	199.2621	233.387	5.836	-26.981
Amentoflavone	176.5495	202.902	-2.314	-18.120
DorsilurinE	162.0289	195.398	-7.930	-17.266
Euchrenone a ¹¹	162.4817	176.775	-16.253	2.503
Cytionic acid A	219.9776	263.069	-3.168	-19.231
Cytionic acid B	217.8486	234.665	11.648	-7.886
Umifenovir	84.8122	0.697	104.886	0.786

MIFS, MIHB, MIES, MUVW: Molecular interaction energy value of the final (optimized) structure, hydrogen bonding, electrostatic and van der Waals interaction (unit: kcal mol⁻¹).

of carboxy compounds affects the inhibition, and nitrogen groups bound with acidic amino acids at the ACE-2 contact site. Umifenovir having one dimethylamino group contacted tightly with ACE-2 via electrostatic interaction, presenting the high MIES value as given in Table 1. Other compounds having amino groups were also tightly bound with ACE-2. These calculated data are not given because they may not be inhibitor candidates.

The additional inhibitor candidates were studied. The compounds are baricitinib [13], calcitriol [14], dexamethasone [15], ivermectin [16,17], losartan [18], N-chloroacetyl-'-sulfonamide-piperazine, N-chloroacetyl-piperidiny-4-carboxamide [19], gallocatechin gallate, amentoflavone [20], dorsilurin E, euchrenone a11 [21], cytoic acid A and B [22], and umifenovir [23]. However, these compounds adsorbed at the ACE-2 contact site and may block the ACE-2 enzyme activity. Their major interaction force was hydrogen bonding that the bonding can be replaced by compounds that should bind using ion-ion interaction and may not block the contact of ACE-2 with S-RBD. The calculated MIFS, MIHB, MIES, and MIVW values are summarized in Table 1.

The modification of the phenolic hydroxy group to the carboxy group may avoid the adsorption at the contact site of ACE-2 as previously observed for carboxy compounds [8]. The modified gallocatechin gallate was effectively rejected from ACE-2 at the docking process. The modified PF-07321332, whose cyano group was changed to carboxy group, was also rejected from ACE-2. Further study is required for the proposed compound to predict the toxicity and the docking with SARS-CoV-2 protein whether the new compound may block the multi-plication or not. The latter analysis requires a supercomputer.

4. Conclusion

The transmissible strength of Covid-19 variants depended on the mutated basic amino acids, especially lysine, at the contact site with ACE-2 that controls blood pressure. The increasing number of basic amino acids at the contact site of S-RBD enhanced the binding affinity with ACE-2. The candidate inhibitors for blocking the S-RBS and ACE-2 binding should be acidic compounds repulsed from ACE-2 because several acidic amino acids exist at the ACE-2 contact site. Various compounds were proposed for the medical treatment against Covid-19 disease. The practical medical treatment compounds should not block the ACE-2 activity by the adsorbent at the contact site. Improving the effectiveness of 14 proposed medicines may need the modification to be repulsed from adsorption on the active site of ACE-2.

Credit author statement

This work was done by only T. Hanai; therefore, he is the sole author and responsible for this manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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