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Research Article

In silico Identification of Characteristics Spike Glycoprotein of SARS-CoV-2 in the Development Novel Therapeutic Candidates for COVID-19 Infectious Diseases

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Abstract

Background: The emergence of infectious diseases caused by SARS-CoV-2 has resulted in more than 90,000 infections and 3,000 deaths. The coronavirus spike glycoprotein encourages the entry of SARS-CoV-2 into cells and is the main target of antivirals. SARS-CoV-2 uses ACE2 to enter cells with an affinity similar to SARS-CoV, correlated with the efficient spread of SARS-CoV-2 among humans.

Objective: In the research were performed identification, evaluation, and exploration of the structure of SARS-CoV and SARS-CoV-2 spike glycoprotein macromolecules and their effects on Angiotensin-Converting Enzyme 2 (ACE-2) using *in silico* studies.

Methods: The spike glycoproteins of the two coronaviruses were prepared using the BIOVIA Discovery Studio 2020. Further identification of the three-dimensional structure and sequencing of the macromolecular spike glycoprotein structure using Chimera 1.14 and Notepad++. To ensure the affinity and molecular interactions between the SARS-CoV and SARS-CoV-2 spike glycoproteins against ACE-2 protein-protein docking simulations using PatchDock was accomplished. The results of the simulations were verified using the BIOVIA Discovery Studio 2020.

Results: Based on the results of the identification of the macromolecular structure of the spike glycoprotein, it was found that there are some similarities in characteristics between SARS-CoV and SARS-CoV-2. Protein-protein docking simulations resulted that SARS-COV-2 spike glycoprotein has the strongest bond with ACE-2, with an ACE score of −1509.13 kJ/mol.

Conclusion: Therefore, some information obtained from the results of this research can be used as a reference in the development of SARS-CoV-2 spike glycoprotein inhibitor candidates for the treatment of infectious diseases of COVID-19.

Keywords: COVID-19; SARS-CoV-2; spike glycoprotein; ACE-2, *in silico* study.
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INTRODUCTION

Three types of coronaviruses have caused deadly pneumonia in humans since the beginning of the 21st century, including severe acute respiratory syndrome coronavirus (SARS-CoV), Middle-Eastern respiratory syndrome coronavirus (MERS-CoV), and SARS-CoV-2.[1]

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SARS-CoV appeared in Guangdong province in China in 2002, infecting 8098 people and causing 774 deaths. In 2012, MERS-CoV appeared in the Arabian Peninsula, infecting a total of 2,494 individuals and claimed 858 lives.[2] Recently appeared a coronavirus named SARS-CoV-2 which was discovered in December 2019 in Wuhan, Hubei province of China. SARS-CoV-2 is linked to an ongoing atypical pneumonia outbreak (COVID-19) which has affected more than 90,000 people and killed more than 3,000 people affected in 60 countries.[3] The World Health Organization (WHO)

declared an epidemic SARS-CoV-2 as a public health emergency of international concern on January 30, 2020 .[4]

MERS-CoV is predicted to originate from bats, but the host reservoir that triggers transmission to humans is a camel. Both SARS-CoV and SARS-CoV-2 are closely related and originate from bats and most likely act as reservoir hosts for these two viruses.[5,6] While palm civets and raccoon dogs have been proven to be intermediaries for SARS-CoV zoonotic transmission between bats and humans, intermediate hosts from SARS-CoV-2 are still unknown.[7] Repeated transmission of the coronavirus in humans along with the detection of various coronaviruses in bats, indicates that zoonotic transmission events will continue in the future.[8] Thus, effective drug candidates for the prevention and control of this phenomenon need to be discovered and developed.

In general, coronavirus entry into host cells is mediated by spike glycoproteins that form homotrimers that protrude from the coronavirus surface.[9,10] This spike glycoprotein consists of two functional subunits that are responsible for binding to host cell receptors and fusion of viral and cellular membranes.[11,12] Previous research confirmed that SARS-CoV and SARS-CoV-2 spike glycoproteins were identical based on a complete phylogenetic analysis of the genome.[13-15] Because of the similarities in the components forming these two types of coronavirus, further identification and exploration is needed.

Moreover, the entry of coronavirus into cells and tissues is also mediated by interactions between the spike glycoprotein and the surface of Angiotensin-Converting Enzyme 2 (ACE-2) to infect host cells. SARS-CoV-2 has an affinity that is comparable to SARS-CoV and MERS-CoV in binding to ACE-2.[16,17] Interaction at the active site of ACE-2 can explain part of the efficient transmission of SARS-CoV-2 in humans, as occurs in SARS-CoV and MERS-CoV.[18,19] Efforts to prevent the attachment of SARS-CoV-2 spike glycoproteins to ACE-2 will be able to control COVID-19 infection.

The need to design effective antiviral candidates to prevent COVID-19 infection has increased. Through this research were conducted the identification, evaluation, and exploration of the structure of spike glycoprotein macromolecules of SARS-CoV and SARS-CoV-2, and the effect of their binding on ACE-2. *In silico* studies can be utilized to observe potential components of this coronavirus.[20,21] Specifically, the SARS-CoV-2 spike glycoprotein is considered the main target of coronavirus which acts as a characteristic formation. Therefore, through this research, information is expected to be obtained that can be used as a reference in developing drug compounds for COVID-19 infectious diseases.

MATERIALS AND METHODS

Preparation of Spike Glycoproteins

Macromolecules that were used in this research were the spike glycoproteins of SARS-CoV and SARS-CoV-2 provided by Protein Data Bank (http://www.rcsb.org/pdb) with PDB ID 2GHV[22] and 6VW1,[23] respectively. The preparation of these spike glycoproteins was performed by removing water molecules and native ligands using BIOVIA Discovery Studio 2020.[24]

Three-Dimensional Structure Identification of Spike Glycoproteins

Spike glycoprotein macromolecules of SARS-CoV and SARS-CoV-2 that have been prepared then overlapped three-dimensional structures with the representation through secondary structures to analyze similarities and observe differences from the macromolecules of the spike glycoproteins. This identification process was accomplished using Chimera 1.14 [25] and BIOVIA Discovery Studio 2020.[24]

Sequencing Analysis of Spike Glycoproteins

Subsequent analysis was performed by sequencing the amino acid of the SARS-CoV and SARS-CoV-2 spike glycoprotein macromolecules using Notepad^{++} and BIOVIA Discovery Studio 2020.[24] The amino acid residues responsible for the structure of the spike glycoprotein macromolecules were then evaluated and explored.

Preparation of Angiotensin-Converting Enzyme (ACE-2)

Angiotensin-Converting Enzyme (ACE-2) macromolecules that were used in this research were provided by Protein Data Bank (http://www.rcsb.org/pdb) with PDB ID 2AJF [26]. The preparation of this ACE-2 macromolecules was accomplished by removing native ligands and water molecules using BIOVIA Discovery Studio 2020 [24].

Spike Glycoprotein Effects on Receptor Binding Domain (RBD) of ACE-2

Protein-protein docking simulations were performed between spike glycoproteins of SARS-CoV and SARS-CoV-2 against Angiotensin-Converting Enzyme (ACE-2) macromolecule. Proteins-protein complex types with default clustering RMSD 4.0 Å were selected. The representation of the Connolly dot surface of the molecule into different components including convex, concave, and flat patch was generated using the PatchDock algorithm.[27] The top 10 candidate solutions were optimized, refined, overhauled, and re-selected the side chain interface. This algorithm also changes the orientation of the molecule relative by limiting flexibility in the side chains of interacting surfaces and allowing the movement of small-rigid objects. The simulation results are then analyzed by visualization using BIOVIA Discovery Studio 2020.[24]

RESULTS AND DISCUSSIONS

Understanding the structure of receptor macromolecules is the first step in studying the mechanism of action of coronaviruses in infecting cells and host tissues. The affinity between the SARS-CoV spike glycoprotein and ACE-2 has been shown to correlate with coronavirus transmission and the severity of infectious diseases that occur in humans. Besides, the ability of coronaviruses to involve ACE-2 from different animal species seems to reflect the host's susceptibility to SARS-CoV infection and facilitate the leap of viruses from animals to humans. SARS-CoV-2 uses ACE-2 as an

entry receptor and recognizes it with characteristics that are identical to SARS-CoV.

In this research, the identification, evaluation, and exploration of spike glycoprotein macromolecules from SARS-CoV and SARS-CoV-2 to observe the characteristics of their macromolecular structure through *in silico* studies. Also, observations were made of the molecular interactions formed between the two spike glycoproteins with ACE-2. Spike glycoprotein macromolecules which will be used in this research were prepared by removing water molecules and native ligands using BIOVIA Discovery Studio 2020. The preparation of these macromolecules was made to facilitate the identification process at a later stage.

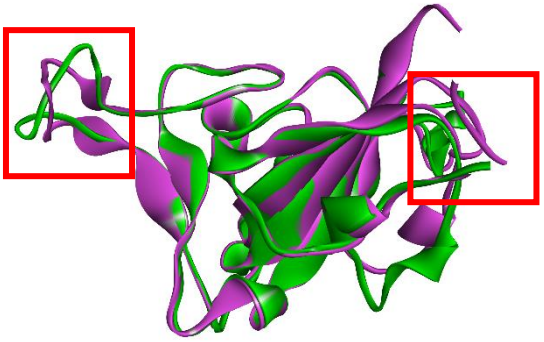

Figure 1. Overlap of three-dimensional structure spike glycoproteins of SARS-CoV (green) and SARS-CoV-2 (purple)

Macromolecules are visualized in the form of three-dimensional conformations to observe and compare between SARS-CoV and SARS-CoV-2 spike glycoproteins through a representation of secondary structures (alpha-helix, beta-sheet, and loop sections). Overall, the two structures of spike glycoprotein macromolecules were identical (Figure 1). However, some parts have differences, as shown in the red square. In these areas, the SARS-CoV spike glycoprotein tends to be in the form of a loop, whereas in SARS-CoV-2 it was in the form of a beta-sheet. This phenomenon shows that there are several different components of amino acid residues that act as the main constituents. Further observation was needed to observe these amino acids, especially those that act as part of the active site of macromolecules.

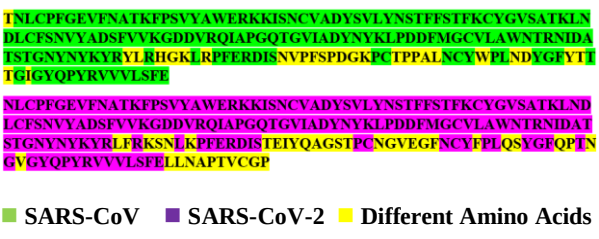

Figure 2. The sequence of spike glycoproteins of SARS-CoV and SARS-CoV-2

After observing based on the three-dimensional structure, it was necessary to explore the sequencing of amino acid residues making up the spike glycoprotein from both coronaviruses. As shown in Figure 2, there are several different amino acids between the SARS-CoV spike glycoprotein and SARS-CoV-2 including Leu445, Phe446, Lys458, Ser459. Asn460, Lys462, Thr470, Glu471, Ile472, Tyr473, Gln474, Ala475, Gly476, Ser477, Asn481, Gly482, Val483, Glu484, Gly485, Phe486, Phe490, Gln493, Ser474, LeN450 Asn519, Ala520, Pro521, Thr522, Val523, Cys524, Gly525, and Pro526 (in the yellow sections). Moreover, the number of glycoprotein amino acid spikes from SARS-CoV-2 was greater than SARS-CoV, which consists of 193. Through this identification, it can be predicted that the SARS-CoV-2 spike glycoprotein will bind more strongly to the surface of ACE-2.

Table 1. The affinity of each spike glycoproteins against ACE-2

Spike glycoprotein	Atomic contact energy (ACE) (kJ/mol)
SARS-CoV	−1305.28
SARS-CoV-2	−1509.13

Further evaluation was accomplished using protein-protein docking simulations. The affinity and molecular interactions that occur between ACE-2 and each spike glycoprotein were observed and compared based on atomic contact energy (ACE) scores integrated into the PatchDock algorithm. The purpose of this docking simulations was to examine the effects of the spike glycoprotein on the active site area of ACE-2 receptor-binding. The spike glycoprotein which has the most amount of amino acid residues was predicted to be able to facilitate the entry of coronavirus into cells and tissues of the human body because of the ability of SARS-CoV and SARS-CoV-2 to reach ACE-2 and forward the infection signaling.

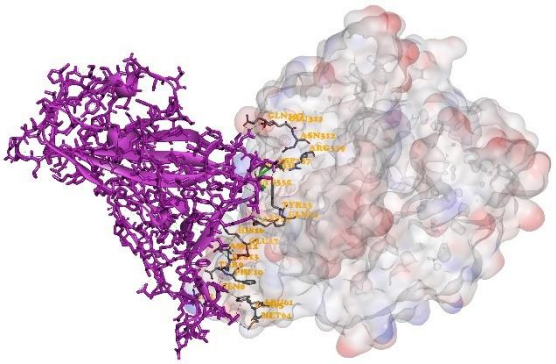

Figure 3. The interactions of SARS-CoV-2 spike glycoproteins (purple) on the surface of ACE-2

Protein-protein docking simulations results in Table 1 show that the spike glycoproteins of SARS-CoV and SARS-CoV-2 have a good affinity with ACE-2, with an ACE score of −1305.28 kJ/mol and −1509.13 kJ/mol, respectively. However, when the results of this simulations were compared, the SARS-CoV spike glycoprotein was able to bind stably to the active site of ACE-2 because it was able to form 23 interactions that include 17 hydrogen bonds (with Arg106, Tyr116, Tyr120, Ala142, Asn154, Tyr156, Gln160, Gly163, Gln165, Thr167, and Gly169), 6 hydrophobic interactions (Leu122, Phe153, Tyr156, and Tyr172), and 2 electrostatic interactions (with Arg106) (Figure 3). Thus, in the effort to design inhibitors for COVID-19,

natural compounds are needed that can inhibit the attachment of spike glycoproteins from SARS-CoV-2 to the ACE-2 surface and stabilize the receptor macromolecular structure and prevent the conformational changes needed to continue further signaling.

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CONCLUSIONS

The results of this research indicate that there are some similarities between the two structures of the spike glycoprotein macromolecules. The spike glycoprotein of SARS-CoV-2 has a fairly strong affinity and interaction with the ACE-2 binding site areas, with an ACE score of −1509.13 kJ/mol. Some information from this research can be used in the development of inhibitor compounds that act as spike glycoprotein inhibitors from SARS-CoV-2. Therefore, it is expected to be able to prevent and control infectious diseases of COVID-19.

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