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Prediction of SARS-CoV-2 3C-like protease (3CL^{pro}) crystal structure to provide COVID-19 inhibitor design through computational studies

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ABSTRACT. Infectious diseases have lately become pandemic, posing a threat to global public health with the introduction of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), previously provisionally named 2019 novel coronavirus or 2019-nCoV). Technological advancements have increased the possibility of discovering natural inhibitor candidates capable of preventing and controlling COVID-19 infections. The SARS-CoV-2 3C-like protease (3CL^{pro}) is critical for SARS-CoV-2 replication and is a prospective therapeutic target. This study aims to identify, evaluate, and explore the 3CL^{pro} macromolecular structures from SARS-CoV and SARS-CoV-2, as well as their impact on angiotensin-converting enzyme 2 (ACE-2). The discovery of the two 3CL^{pro} macromolecules revealed structural similarities in several regions. These findings were subsequently confirmed by performing protein-protein docking simulations to observe the interaction of 3CL^{pro} with the active site ACE-2. With an ACE score of 701.41 kJ/mol, SARS-COV-2 3CL^{pro} forms the strongest binding with ACE-2. As a result, the findings of this research can be used to guide the development of potential SARS-CoV-2 3CL^{pro} inhibitors for the treatment of COVID-19 infectious diseases.

Keywords: angiotensin-converting enzyme 2 (ACE-2), computational study; COVID-19; SARS-CoV-2, 3C-like protease (3CL^{pro})

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INTRODUCTION

At the end of December 2019, a new coronavirus was identified in Wuhan City, Hubei Province, China. It was first named 2019-nCoV (now SARS-CoV-2) (ICTV, 2020). The first proven case of human-to-human transmission occurred in Guangdong, China (Chan *et al*., 2020). On 15 February 2020, the World Health Organization (WHO) proclaimed this outbreak a global public health emergency; there have been over 65000 confirmed cases and over 1500 deaths (Zhou *et al*., 2020). This coronavirus is spreading at a much faster rate and on a much larger scale than the past coronaviral epidemics (SARS-CoV and MERS-CoV) (Shereen *et al*., 2020; Yi *et al*., 2020). This infectious disease has now become a pandemic, impacting over 850000 people and resulting in over 42000 deaths in more than 180 countries or regions (Chen *et al*., 2020).

The coronavirus genome has been shown to be identical to the beta-coronavirus (80% identity) responsible for the severe acute respiratory syndrome (SARS), which originated in bats and caused a global outbreak in 2003 (Guzzi *et al*., 2020; Mohammad *et al*., 2020; Zheng, 2020). Appropriate research is now gaining momentum in the development of antiviral medicines to treat SARS-CoV. Until now, there has not been yet treatment for SARS (Yao *et al*., 2020). However, the knowledge gathered via intensive research and development activities may aid in the creation of current therapeutic alternatives.

The coronavirus genome encodes more than 20 proteins, including two proteases (PL^{pro} and 3CL^{pro}) required for virus replication and transcription (Prajapat *et al*., 2020). The two polyproteins (PP1A and PP1AB) are subsequently cleaved into their separate functional components by this protease (Lu *et al*., 2020). The 3-chymotrypsin-like protease (3CL^{pro}) is considered a target in developing promising drugs. This extraordinary effort has

been made to study the structure of $3CL^{pro}$ to develop therapies for SARS-CoV and other pathogenic coronaviruses, such as the Middle-East respiratory syndrome coronavirus (MERS-CoV) and COVID-19 (SARS-CoV-2), since it shares active sites and enzymatic mechanisms (Pachetti *et al*., 2020).

Coronavirus enters cells and tissues mediated by $3CL^{pro}$ which is found on the surface of the coronavirus and can bind directly to Angiotensin-Converting Enzyme 2 (ACE-2) to infect host cells (Liu *et al*., 2020). The binding affinity of SARS-CoV-2 to ACE-2 is comparable to SARS-CoV and MERS-CoV (Graham *et al*., 2012). Strong bonding on the surface of ACE-2 can explain part of the efficient transmission of SARS-CoV-2 in humans, as occurs in SARS-CoV and MERS-CoV (Ng *et al*., 2016; Liu *et al*., 2017). Inhibition of $3CL^{pro}$ adhesion to SARS-CoV-2 with ACE-2 is expected to prevent COVID-19 infection.

At the current epidemic, it is critical to develop a new candidate virus that is effective against coronaviruses, particularly SARS-CoV-2. As our previous studies have constantly concentrated on computational investigations of SARS-CoV-2 (Fakih, 2020a; Fakih *et al*., 2020b; Ramadhan *et al*., 2020; Ramadhan *et al*., 2021). This study aims to identify, evaluate, and explore the $3CL^{pro}$ macromolecular structure of SARS-CoV and SARS-CoV-2, and their affinity for ACE-2. Computational investigations can be utilized to observe $3CL^{pro}$, a possible coronavirus component (Kumar *et al*., 2020). In particular, $3CL^{pro}$ from coronavirus is considered a target since it plays a critical role in the development of this virus's characteristics. It is anticipated that this research will provide some information to aid in the development of candidates for novel therapeutic compounds for COVID-19 diseases.

MATERIALS AND METHODS

Preparation of macromolecule structure. The crystal structure of the $3CL^{pro}$ macromolecules used in this study was obtained from the Protein Data Bank (https://www.rcsb.org/) with PDB ID 2PWX ($3CL^{pro}$ SARS-CoV) and 6M2N ($3CL^{pro}$ SARS-CoV-2). Preparation of SARS-CoV $3CL^{pro}$, SARS-CoV-2 $3CL^{pro}$, and ACE-2 macromolecules was performed by removing water molecules and natural ligands using BIOVIA Discovery Studio 2020 (Berman *et al*., 2020; BIOVIA, 2020; Verma *et al*., 2021).

Three-dimensional conformation analysis of $3CL^{pro}$ macromolecules. The $3CL^{pro}$ macromolecular structure of the prepared SARS-CoV and SARS-CoV-2 then overlaps the three-dimensional conformation with the representation of the secondary structure to observe similarities and identify the differences between the two $3CL^{pro}$ coronavirus macromolecules. This stage was accomplished using BIOVIA Discovery Studio 2020 and Chimera 1.14 (BIOVIA, 2020; Alamri *et al*., 2021; Rajpoot *et al*., 2021).

Sequencing identification of $3CL^{pro}$ macromolecules. Further identification was performed on the sequencing of both the SARS-CoV $3CL^{pro}$ and SARS-CoV-2 $3CL^{pro}$ macromolecular structures using BIOVIA Discovery Studio 2020 and Notepad ++. The amino acid residues that were responsible for the main components of $3CL^{pro}$ macromolecules were then evaluated and explored (Tomic *et al*., 2020; Verma *et al*., 2021).

Protein-protein docking simulations. Protein-protein docking simulations were accomplished using the PatchDock algorithm to identify the affinity and molecular interactions between the structure of the SARS-CoV $3CL^{pro}$ and SARS-CoV-2 $3CL^{pro}$ macromolecules against ACE-2 (Schneidman-Duhovny *et al*., 2005; Ansari *et al*., 2020). The surface distance of macromolecules was limited to a maximum radius of 4.0 Å. The parameters used in this simulation were based on the representation of the shape of the molecule, the active site of the target receptor, and the selection and assessment. These docking simulations were conducted efficiently without rigid molecular bonds.

Analysis of protein-protein docking simulations. The affinity of protein-protein was analyzed and compared based on the atomic contact energy (ACE) score. Then

further observations were made on amino acid residues that play an important role in protein-protein interactions using the BIOVIA Discovery Studio 2020 (BIOVIA, 2020; Camacho *et al*., 2000; Banerjee *et al*., 2021).

RESULTS AND DISCUSSION

The introduction of a macromolecular structure is the first step in understanding the mechanism of action of coronavirus in infecting cells and host tissues. The strong affinity and interaction between SARS-CoV $3CL^{pro}$ and ACE-2 have been linked to increased coronavirus transmission and the severity of infectious diseases in humans (Sasidharan *et al*., 2020; Zhang *et al*., 2021). The ability of the coronavirus to involve the surface of ACE-2 from different animal species seems to reflect the susceptibility of the host to SARS-CoV infection and facilitate the leap in coronavirus from animals to humans. Similar to SARS-CoV, SARS-CoV-2 uses ACE-2 as an entry receptor and recognizes it with identical affinity (Alipoor *et al*., 2021; Rotondi *et al*., 2021).

Through this research, the identification, evaluation, and exploration of $3CL^{pro}$ macromolecules from SARS-CoV and SARS-CoV-2 observe the similarities and differences in the structure of macromolecules that describe the structural characteristics of its constituent components through computational studies. Additionally, molecular interactions between the two $3CL^{pro}$ macromolecules and ACE-2 was also observed. Using BIOVIA Discovery Studio 2020, macromolecules for this investigation were prepared by eliminating water molecules and natural ligands. Macromolecular preparations were made to facilitate the process of identification and evaluation in subsequent procedures.

An identification of the $3CL^{pro}$ macromolecular visualization was made with the representation of the secondary structure (alpha-helix, beta-sheet, and loop section) to allow for observation the entire structure of macromolecule structure. Several $3CL^{pro}$ components from SARS-CoV and SARS-CoV-2 were identical, as illustrated in Fig. 1. However, there was a slight difference at the edges of the two $3CL^{pro}$ macromolecules (orange box section). The end part of the $3CL^{pro}$ SARS-CoV ends with an alpha-helix shape, whereas the $3CL^{pro}$ SARS-CoV was slightly longer forms a loop. This phenomenon demonstrates that the $3CL^{pro}$ SARS-CoV macromolecule contained some additional amino acid residues at the end. Further observation is needed by comparing the sequences of the two $3CL^{pro}$ macromolecules to explore amino acid residues that play an important role as a major component.

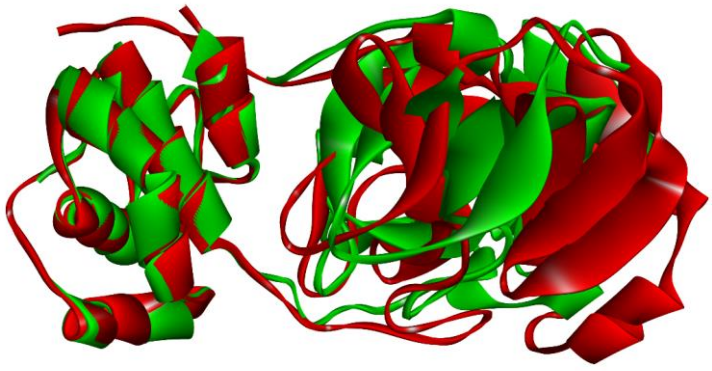

Fig. 1. Overlaps the three-dimensional conformation SARS-CoV $3CL^{pro}$ (green) and SARS CoV-2 (red).

Besides observing the three-dimensional structure, further exploration of the sequencing of $3L^{pro}$ of the two coronaviruses was also needed. Fig. 2 shows that there were several different amino acids between $3CL^{pro}$ SARS-CoV and SARS-CoV-2 including Ser1, Gly2, Phe3, Gly11, Val35, Ser46, Asn65, Val86, Lys88, Ala94, Phe134, Asp155, Asn180, Val202, Val202, Val35 Arg222, Phe223, Thr224, Ser267, Asn277, Gly278, Arg279,

Ala285, Leu286, Cys300, Ser301, Gly302, Val303, Thr304, Phe305, and Gln306 (yellow section). Then, the amount of 3CL^{pro} amino acid residues from SARS-CoV-2 was greater than SARS-CoV, consisting of 306. Through this observation, it can be predicted that SARS-CoV-2 3CL^{pro} will have a strong affinity and interaction to the surface of ACE-2 compared to SARS-CoV 3CL^{pro}.

RKMAFPSAKVEGCMVQVTCGTTTLNGLWLDDTVYCPRHVICTAEDMLNPNYEDLLIRKSNHSFL
VQAGNVQLRVIGHSMQNCLLRLKVDTSNPKTPKYKFVRIQPGQTFSVLACYNGSPSGVYQCAMRP
NHTIKGSFLNGSCGSVGFNIDYCVSFCYMHHMELPTGVHAGTDLEGKFYGPFVDRQTAQAAGTDT
TITLNVLAWLYAAVINGDRWFLNTTLNDFNLVAMKYNYEPLTQDHVDILGPLSAQTGIAVLDMC
AALKELLQNGMTILGSTILEDEFTPFDVVRQ

SGFRKMAFPSGKVEGCMVQVTCGTTTLNGLWLDDVVYCPRHVICTSEDMLNPNYEDLLIRKSNH
NFLVQAGNVQLRVIGHSMQNCVLKLKVDTANPKTPKYKFVRIQPGQTFSVLACYNGSPSGVYQCA
MRPNFTIKGSFLNGSCGSVGFNIDYDCVSFCYMHHMELPTGVHAGTDLEGNFYGPFVDRQTAQAA
GTDTTITVNVLAWLYAAVINGDRWFLNRFTTTLNDFNLVAMKYNYEPLTQDHVDILGPLSAQTGI
AVLDMCASLKELLQNGMNGRTILGSALLEDEFTPFDVVRQCSGVTFQ

Fig. 2. The sequencing of SARS-CoV 3CL^{pro} (green) and SARS-CoV-2 3CL^{pro} (red) macromolecular structure.

Protein-protein docking simulations were accomplished to observe the 3CL^{pro} affinity of both coronaviruses against ACE-2. This binding affinity was evaluated by the atomic contact energy (ACE) score which is integrated into the PatchDock algorithm. This docking simulation was performed to evaluate the effect of the active site binding of 3CL^{pro} on the surface of ACE-2. The 3CL^{pro} binding area was predicted to facilitate the entry of coronavirus into cells and host tissues because of the ability of SARS-CoV and SARS-CoV-2 to achieve ACE-2 in continuing signaling. Exploration of amino acid residues was also needed that plays an important role in the formation of molecular interactions between 3CL^{pro} coronavirus and ACE-2.

Table 1. The affinity of SARS-CoV 3CL^{pro} and SARS-CoV-2 3CL^{pro} against ACE-2.

3CL^{pro}	Atomic contact energy (kJ/mol)
SARS-CoV	−540.66
SARS-CoV-2	−701.41

According to protein-protein docking simulations in Table 1, SARS-CoV-2 3CL^{pro} has a stronger affinity for ACE-2 than SARS-CoV 3CL^{pro}, with an ACE score of -701.41 kJ/mol and -540.66 kJ/mol, respectively. This phenomenon may be explained by the fact that SARS-CoV-2 3CL^{pro} is larger in size, containing 306 amino acid residues. Moreover, there were additional amino acids at the end of the SARS-CoV-2 3CL^{pro} sequence, including Ser1, Gly2, Phe3, Cys300, Ser301, Gly302, Val303, Thr304, Phe305, and Gln306.

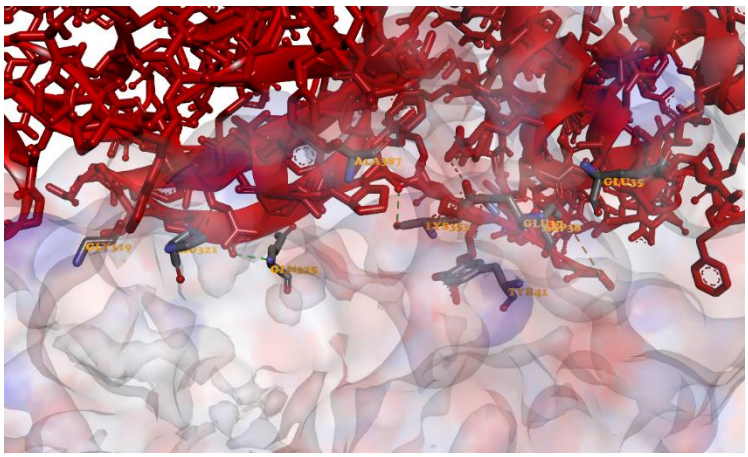

Fig. 3. Molecular interaction between SARS-CoV-2 3CL^{pro} (red) and the surface area of ACE-2.

Furthermore, ten connections can be formed between SARS-CoV-2 $3CL^{pro}$ and the surface portion of ACE-2, including four hydrogen bonds (with Glu35, Gly319, Gln325, and Lys353), four hydrophobic interactions (with Tyr41, Pro321, and Ala387), and two electrostatic interactions (with Glu37 and Asp38) (Fig. 3). Thus, efforts must be made to develop COVID-19 inhibitors capable of inhibiting $3CL^{pro}$ adhesion from SARS-CoV-2 to ACE-2, stabilizing the structure of receptor macromolecules, and preventing the conformational changes required for signaling to continue.

CONCLUSION

The two $3CL^{pro}$ coronaviruses shared some similarities. With an ACE score of 701.41 kJ/mol, SARS-CoV-2 $3CL^{pro}$ exhibits a strong affinity and interaction in the area of the binding site ACE-2. This study's findings are expected to aid in the development of inhibitor compounds that serve as $3CL^{pro}$ inhibitors of SARS-CoV-2. As a result, infectious diseases caused by COVID-19 can be prevented and treated.

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