Conference Paper

The Inhibition of Angiotensin-Converting Enzyme 2 Receptors of SARS-CoV-2 Through Mucroporin Derived from Scorpion Venom

Taufik Muhammad Fakih*, Mentari Luthfika Dewi and Eky Syahroni

Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Islam Bandung, Bandung, Indonesia

ORCID ID
Taufik Muhammad Fakih : https://orcid.org/0000-0001-7155-4412
Mentari Luthfika Dewi : https://orcid.org/0000-0002-9192-3163
Eky Syahroni : https://orcid.org/0000-0003-1126-4418

Abstract.
The SARS-CoV-2 virus that causes COVID-19 has a spike glycoprotein that can bind to a host cell receptor, angiotensin-converting enzyme 2 (ACE-2). This plays an important role in the entry of viral cells. Therefore, targeting of the ACE- 2 receptor holds promise as a potential target for anti-viral interventions to prevent and inhibit COVID-19. This study aims to focus on in silico studies to screen alternative drugs that can block ACE-2 receptor properties as a receptor for SARS- CoV-2. It is a potential therapeutic target for COVID-19 using the bioactive peptide Mucroporin which is derived from scorpion venom. There were four sequences of Mucroporin peptides modeled using the PEP-FOLD 3.5 server. The protein- peptide-based molecular docking simulations were used to identify and evaluate the actions of Mucroporin against ACE-2 receptors using PatchDock. The best response is then further observed using BIOVIA Discovery Studio 2020. This study revealed that Mucroporin and Mucroporin-S1 gave the best docking scores compared to Mucroporin-M1 and Mucroporin-S2, with the binding free energy values of −943.53 kJ/mol, −162.42 kJ/mol, 867.80 kJ/mol and 43.14 kJ/mol respectively. This study reveals for the first time that Mucroporin and Mucroporin-S1 are functional inhibitors of ACE-2 and as such, that components of scorpion venom can be used as potential inhibitors to the ACE-2 receptor of SARS-CoV-2.

Keywords: SARS-CoV-2; COVID-19; Angiotensin-Converting Enzyme 2 (ACE-2); Mucroporin; In Silico Study

Corresponding Author: Taufik Muhammad Fakih; email: taufikmuhammadf@gmail.com

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1. INTRODUCTION

Coronaviridae is a large family of viruses that can cause disease in mammals. In humans, this virus can cause a variety of illnesses from the common cold to severe respiratory illnesses. In 2002, severe acute respiratory syndrome (SARS) emerged, while in 2012, the Middle East respiratory syndrome (MERS) emerged. Both viruses are beta coronaviruses that are transmitted from animals to humans and can cause severe respiratory disease in infected individuals [1]. In December 2019 in China, appeared

SARS-coronavirus-2 (SARS-CoV-2) also known as the 2019- novel coronavirus (2019-nCoV) or coronavirus-2019 (COVID-19), causing an outbreak of pneumonia and then extends throughout the world [2]. Recently, the first genome sequence for COVID-19 was released, and through comparisons with the SARS-CoV and MERS-CoV genomes, it was found that COVID-19 has better genome sequence homology compared to SARS-CoV and MERS-CoV [3,4].

Since the SARS-CoV outbreak was first discovered, it has been identified that angiotensin-converting enzyme 2 (ACE-2) is used by SARS-CoV as a receptor for entry into human host cells [5]. Overexpression of ACE-2, caused by the attachment of the SARS-CoV spike protein, can block the renin-angiotensin pathway [6]. Recent studies have revealed that the COVID-19 protein spike has a strong affinity for ACE-2 in host cells and is significantly higher than SARS-CoV [7,8]. Therefore, ACE-2 inhibitors could be potential targets for the development of anti-virus candidates. Another study also reported that the COVID-19 receptor-binding domain was able to enter cells expressing human ACE-2, while other receptors were ineffective, confirming that human ACE-2 is the primary receptor for COVID-19 [9]. Since host cell receptors play an important role in virus entry, targeting ACE-2 receptors is a promising strategy in the prevention of COVID-19 infection.

Until now, no vaccine or definite treatment has been developed for the coronavirus that causes COVID-19. Nevertheless, many possible treatments for COVID-19 have been under the spotlight by scientists and the healthcare industry. For example, a combination of the antimalarial drugs chloroquine and hydroxychloroquine as well as the anti-HIV drugs ritonavir and lopinavir have been recommended [10,11]. Because these drugs directly target pathogens, their effectiveness is largely temporary. Besides, the development of new drugs to target ACE-2 and treat COVID-19 could take a while. Therefore, the safety efficacy of new drugs is a major concern in the development of anti-COVID-19 candidates. Also, traditional medicine from many geographic areas using herbal medicine can be an alternative option for viral infections, including those caused by SARS-CoV [12,13].

Interestingly, among several natural product sources that are predicted to be agents of the treatment of COVID- 19, animal venom has revealed great potential [14]. Although there is a dangerous mechanism of action of this animal venom, it has components that play an important role as medicine to cure certain diseases. It has been widely reported in various kinds of literature that animal venoms are rich in antimicrobial substances and contain various biologically active compounds with various chemical structures [15,16]. One of them is antimicrobial peptides (PAM) which are a group of peptides with

an important function in the innate immune response when attacked by pathogenic organisms, such as fungi, bacteria, and viruses which are considered as the first line of defense of most organisms, including plants, insects, and vertebrates [17–20].

Mucroporin derived from scorpion venom (*Lychas mucronatus*) is one of the PAMs which has activity against SARS-CoV and influenza H5N1 [21–25]. Through this research, we will observe the molecular interactions between Mucroporin and ACE-2. The computational approach using in silico studies can be used to identify, evaluate, and explore the affinity of Mucroporin as a potential inhibitor of ACE-2 [26]. Thus, from this research, it is hoped that the molecular structure of Mucroporin will be obtained as a candidate in the treatment of COVID-19 infection.

2. METHODS

In this research, the method used is the in silico study by utilizing a peptide-protein docking simulation. This research aims to observe the affinity and molecular interactions between the antimicrobial peptide molecules Mucroporin derived from the scorpion venom (*Lychas mucronatus*) against the ACE-2 macromolecule. Some of the peptide molecules to be used were modeled using the PEP-FOLD 3.5 server and ACE-2 macromolecules were also prepared before use using MGLTools 1.5.6 with AutoDock 4.2 software. Then an interaction study was performed using PatchDock software between Mucroporin molecules and ACE-2 macromolecules. The results of the peptide- protein docking simulation are then identified and compared based on the atomic contact energy (ACE) score. In addition, exploration and evaluation of molecular interactions that are formed using the BIOVIA Discovery Studio 2020 software are also demonstrated.

2.1. Software, Hardware, and Materials

In this research, the software used is the Windows 10 Operating System and Linux Ubuntu 20.04, MGLTools 1.5.6 with AutoDock 4.2, PEP-FOLD 3.5 server, PatchDock, and BIOVIA Discovery Studio 2020. In this research, the hardware used is a computer with the specifications of the Intel (R) Core i3-6100 CPU @ 2.30GHz (4 CPUs), 4096 MB RAM, 320GB hard drive, and VGA Intel HD Graphics 520. In this research, the material used was the ACE-2 macromolecular crystal structure. The target macromolecules were obtained from the Protein Data Bank with the PDB ID 6VW1 and have a resolution of 2.68 Å (Figure 1) [27]. In this research, the antimicrobial peptide molecules used were Mucroporin peptides and their derivatives (Mucroporin-M1, Mucroporin-S1, and

Mucroporin-S2) derived from scorpion venom (*Lychas mucronatus*) and have been shown to have activity against SARS-CoV and influenza H5N1 in previous studies [28,29].

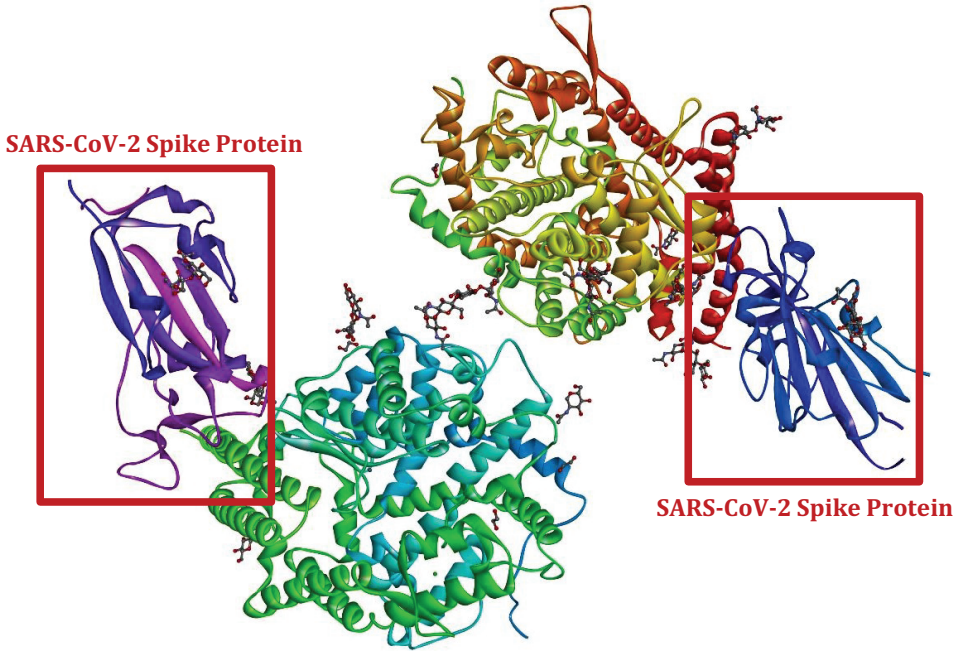

Figure 1: The macromolecular structure of angiotensin-converting enzyme 2 (ACE-2) which forms a complex with the SARS- CoV-2 spike protein.

2.2. Procedures

2.2.1. Preparation of Angiotensin-Converting Enzyme 2 (ACE-2) Macromolecules

The macromolecular crystal structure of the ACE-2 that has been downloaded from the Protein Data Bank is then prepared first using MGLTools 1.5.6 with AutoDock 4.2 software. This protein macromolecule preparation is performed by removing water molecules and natural ligands, followed by adding polar hydrogen atoms and calculating Kollman's partial charge [30].

2.2.2. Modelling of Mucroporin Peptide Molecules

Molecular modeling of the Mucroporin antimicrobial peptide was performed using the PEP-FOLD 3.5 server (Figure 2). The PEP-FOLD 3.5 server is a software used to model the sequencing of peptide molecules with the number of amino acids between 5 and 50 into three-dimensional (3D) conformations using the de novo method [31]. The peptide molecule that has been modeled then chooses the best conformation based on the

energy value of the SOPEP (Optimized Potential for Efficient Structure Prediction), added polar hydrogen atoms, and Kollman's partial charge was calculated [32].

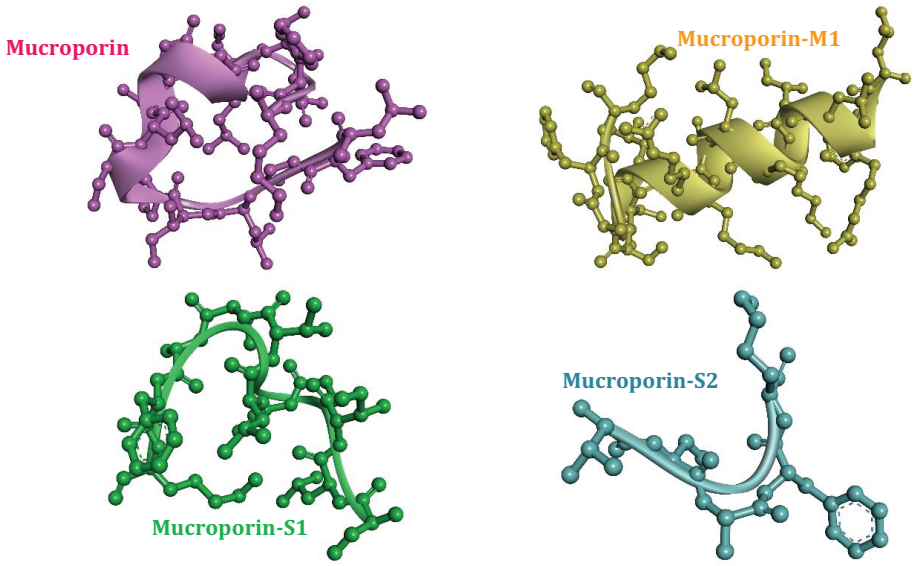

Figure 2: The three-dimensional structure of the antimicrobial peptide molecule Mucroporin and its derivatives.

2.2.3. The Simulation of Protein-Peptide Docking

A protein-peptide docking simulation was performed using PatchDock software to identify and evaluate the affinity between the antimicrobial peptide molecule Mucroporin and ACE-2 macromolecules. The distance between the Mucroporin antimicrobial peptide molecule and the macromolecular surface of the ACE-2 is limited to a maximum radius limit of 2.0 Å. This molecular docking simulation uses parameters based on the representation of the shape of the molecule, the active site area of protein macromolecule binding, and selection with an assessment. This molecular docking simulation is also performed efficiently without any rigid bonds between molecules [33].

2.2.4. The Analysis of Protein-Peptide Docking Simulation Results

The results of the peptide-protein docking simulation were then performed observation and exploration of the molecular interactions that formed between the Mucroporin antimicrobial peptide molecule and the ACE-2 macromolecules based on the atomic contact energy (ACE) score [34]. The amino acid residues that are responsible for the formation of molecular interactions were then observed using the BIOVIA Discovery Studio 2020 software [35].

3. RESULTS AND DISCUSSIONS

Currently, vaccines and therapeutic candidates that have good activity with minimum side effects are needed to prevent and treat the COVID-19 infectious disease, which until now has become a pandemic. Antimicrobial peptides can be considered an ideal choice in antiviral therapy because of their properties and characteristics that are more specific to the target protein. Research showing antimicrobial peptides from multiple sources has so far increased. However, there are few data regarding the specificity of their inhibitory ability. Thus, further observation steps are needed to ascertain the antiviral potential of these antimicrobial peptides.

Previous studies revealed that the antimicrobial peptide Mucroporin derived from the scorpion venom (*Lychas mucronatus*) has antiviral activity against SARS-CoV and influenza H5N1 [21–25]. This research will predict the affinity and molecular interactions of the antimicrobial peptide Mucroporin against angiotensin-converting enzyme 2 (ACE-2) through in silico using a peptide-protein docking simulation.

The macromolecular structure of ACE-2 was selected as the target protein for the antimicrobial peptide molecule Mucroporin. The protein macromolecules were prepared beforehand by removing water molecules and natural ligands, then adding polar hydrogen atoms, and Kollman's partial charge was calculated using MGLTools 1.5.6 with AutoDock 4.2 software [30]. This protein macromolecule preparation is performed to ensure that the Mucroporin antimicrobial peptide molecule can interact stably with a good affinity for the active site of the protein macromolecule.

TABLE 1: Energy value of the sOPEP (Optimized Potential for Efficient Structure Prediction) of the antimicrobial peptide molecule Mucroporin.

Antimicrobial Peptide Molecule	Peptide Molecule Sequence	sOPEP Energy Value
Mucroporin	LFGLIPSLIGGLVSAFK	–27.90
Mucroporin-M1	LFRLIKSLIKRLVSAFK	–36.43
Mucroporin-S1	SLIGGLVSAFK	–14.33
Mucroporin-S2	VSAFK	–2.44

Sequencing modeling of the antimicrobial peptide Mucroporin into three-dimensional (3D) structures was performed using the PEP-FOLD 3.5 server. Based on the results of conformational modeling, the best results are selected based on the energy value of the SOPEP (Optimized Potential for Efficient Structure Prediction) [36–38]. This energy value of sOPEP has been integrated into the PEP-FOLD server so that it can be used to predict the conformation of the peptide molecular structure being modeled to be close to the actual state and be able to interact with the active site area of the target protein

macromolecule in a stable manner. Based on the modeling results of the antimicrobial peptide molecule Mucroporin in Table 1, it can be predicted that the peptide is able to form a good affinity for the ACE-2 macromolecules.

TABLE 2: The atomic contact energy (ACE) score between the antimicrobial peptide molecule Mucroporin and the angiotensin-converting enzyme 2 (ACE-2) macromolecules.

Antimicrobial Peptide Molecule	Peptide Molecule Sequence	ACE score (kJ/mol)
Mucroporin	LFGLIPSLIGGLVSAFK	–943.53
Mucroporin-M1	LFRLIKSLIKRLVSAFK	867.80
Mucroporin-S1	SLIGGLVSAFK	–162.42
Mucroporin-S2	VSAFK	43.14

In silico studies using peptide-protein docking simulations were performed using PatchDock software to observe the best affinity and molecular interactions among the four antimicrobial peptide molecules Mucroporin against ACE- 2 macromolecules. The best conformation from the molecular docking simulation results was selected based on the PatchDock value and compared using the atomic contact energy (ACE) score [34]. Based on the results of the molecular docking shown in Table 2, it is predicted that the antimicrobial peptides Mucroporin and Mucroporin-S2 have better affinity when compared to other antimicrobial peptides, with the ACE scores of –943.53 kJ/mol and –162.42 kJ/mol, respectively.

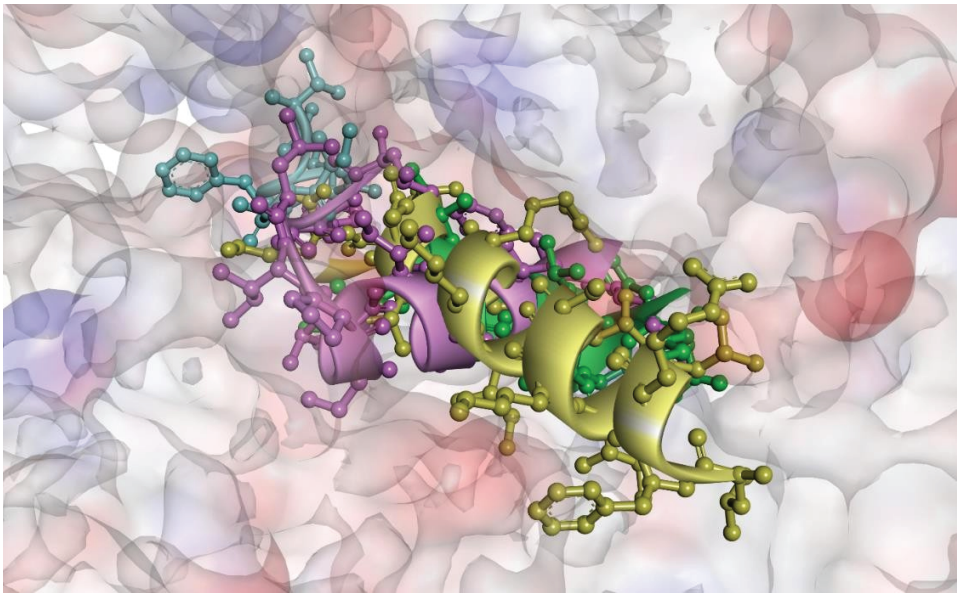

Figure 3: The binding conformation of the antimicrobial peptide molecules Mucroporin (red), Mucroporin-M1 (yellow), Mucroprin-S1 (green), and Mucroporin-S2 (blue) on the active site of the ACE-2 macromolecule.

As shown in Fig. 3, overall the four molecules of the antimicrobial peptide Mucroporin occupy the polar part of the ACE-2 macromolecule. This phenomenon can occur

because the PatchDock software algorithm simulates molecular docking without rigid intermolecular bonds so that the Mucroporin antimicrobial peptide molecule can occupy part of the ACE-2 macromolecule based on its flexibility. The best affinity shown by the antimicrobial peptide molecule Mucroporin can be due to the many molecular interactions formed with ACE-2 macromolecules which include four hydrogen bonds (with Thr276, Pro346, Glu406, and Thr445), eight hydrophobic interactions (with Trp271, Phe274, His345, Met366, Leu370, His374, Ala413, and Tyr515), and two electrostatic interactions (with Asp367 and Glu402) (Table 3).

TABLE 3: Molecular interactions between the antimicrobial peptide molecule Mucroporin and the ACE-2 macromolecule.

Antimicrobial Peptide Molecule	Number of Interactions	Amino Acid Residues
Mucroporin	14	Glu402, Asp367, Pro346, Thr276, Thr445, Glu406, His374, Tyr515, Ala413, Met366, Leu370, Trp271, Phe274, His345
Mucroporin-M1	13	Asp367, Asp292, Glu150, Glu145, Tyr515, Arg518, Phe504, Ala153, Phe274, His345, His374, Phe504, His505
Mucroporin-S1	9	Asp292, Asp367, Thr276, Asn290, Thr445, Ser409, Pro346, Met366, Ala413
Mucroporin-S2	5	Arg514, Tyr515, His401, His345, Phe504

Then the antimicrobial peptide molecule Mucroporin-S1 can form nine interactions with ACE-2 macromolecules consisting of three hydrogen bonds (with Thr276, Asn290, and Thr445), four hydrophobic interactions (with Pro346, Met366, Ala413, and Ser409), and two electrostatic interactions (with Asp292 and Asp367). However, the positive ACE scores of the antimicrobial peptide molecules Mucroporin-M1 and Mucroporin-S2 were predicted due to undesirable interactions with the active sites of ACE-2 macromolecules. Based on this phenomenon, the antimicrobial peptide molecules Mucroporin and Mucroporin-S1 can be predicted to prevent the attachment of the SARS-CoV-2 spike protein on the surface of the ACE-2 receptor so that it is unable to infect human host cells [39,40].

4. CONCLUSIONS

The antimicrobial peptide molecules Mucroporin and Mucroporin-S1 can inhibit ACE-2 macromolecules through identification, evaluation, and exploration of affinity and molecular interactions formed through the in silico peptide- protein docking method.

Based on the simulation results of molecular docking, it was found that the antimicrobial peptide molecules had ACE scores of –943.53 kJ/mol and –162.42 kJ/mol, respectively. Thus, these antimicrobial peptides have potential as candidates for ACE-2 inhibitors in the development of peptide-based treatment of COVID- 19 infectious diseases.

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