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Dermaseptin-Based Antiviral Peptides to Prevent COVID-19 through *In Silico* Molecular Docking Studies against SARS-CoV-2 Spike Protein

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ABSTRACT

A pandemic coronavirus disease of 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has now been declared a global pandemic by the World Health Organization. The search for new drugs, especially by utilizing antiviral peptides is a very potential area. Through this study, protein-peptide docking and protein-protein docking simulations were conducted using *in silico* methods to identify, evaluate, and explore the molecular affinity and interaction of dermaseptin peptide molecules produced by frogs of the genus *Phyllomedusa* against the SARS-CoV-2 spike protein macromolecule, and its effect on attachment to the surface of the ACE-2 (Angiotensin Converting Enzyme-2) receptor. Protein-peptide docking simulation results show that dermaseptin-S9 peptide molecule has the best affinity to the active site of SARS-CoV-2 spike protein macromolecule binding site, with a binding free energy value of −792.93 kJ/mol. Then the results of protein-protein docking simulations proved that dermaseptin-S9 peptide molecule was able to prevent the attachment of SARS-CoV-2 spike protein to the surface of the ACE-2 receptor, with a total energy value of 517.85 kJ/mol. Therefore, it is hoped that dermaseptin-S9 peptide molecule can be further studied in the development of novel antiviral peptide candidates for the control of COVID-19 infectious disease.

Keywords: *COVID-19; SARS-CoV-2 spike protein; dermaseptin; antiviral peptide; in silico study*

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INTRODUCTION

The World Health Organization has now declared a global emergency and a pandemic for novel coronavirus disease of 2019 (COVID-19, formerly called 2019-nCoV) which has been actively spreading throughout the world. The COVID-19 infectious disease caused by the SARS-CoV-2 virus can cause symptoms such as fever, cough, pneumonia, nausea, and fatigue. To date, SARS-CoV-2 has spread to almost 24 countries worldwide and more than 8,061,550 people have been reported to be infected by 17 June 2020. Among these, there have been 440,290 deaths reported to be related to COVID-19 (Gabutti et al., 2020).

The main epidemiological cause of the SARS-CoV-2 virus is thought to originate from the seafood market in Wuhan City, Hubei Province, China (Chen et al., 2020). However, the true center of the initial transfer to humans is still unknown. At present, there are more than 100 complete genome sequences known in NCBI GenBank obtained from approximately 10 countries. Later it was also found that the variation between these sequences was less than 1% (Lu et al., 2020; Sah et al., 2020).

The SARS-CoV-2 virus is closely related to SARS-CoV and this allows the use of a known protein structure to quickly study models to find candidate compounds in the prevention and treatment of this SARS-CoV-2 virus (Hui et al., 2020). While traditional methods of drug discovery can take years, the approach that can be utilized here to find a predictable drug for SARS-CoV-2 is to use *in silico* protein-peptide docking studies of the most variable target proteins in SARS-CoV-2, which is spike protein from SARS-CoV-2 (Tahir ul Qamar et al., 2020; Wu et al., 2020).

SARS-CoV-2 spike protein is responsible for controlling some of the main functions of the virus and has a catalytic domain that is highly conserved from the SARS-CoV virus (Zhang et al., 2020). Some other functions include the virus replication process which makes it an ideal target in drug development (Das et al., 2020; Ton et al., 2020). It has been computationally proven that SARS-CoV-2 has a mechanism that is identical to the SARS-CoV virus and has a high affinity for ACE-2 (Angiotensin Converting Enzyme-2) receptor (Xu et al., 2020). Besides, there are structural similarities between SARS-CoV-2 and SARS-CoV spike proteins, which conservation is only 73% with most of the variability in the area of host cell protein interaction (Hall & Ji, 2020).

Dermaseptin is an antiviral peptide produced by frogs of the genus *Phyllomedusa*. The antiviral activity of this peptide has been described for HSV-1, HSV-2, and HIV-1, in which the virus envelope appears to be the

preferred target of dermaseptin (Bergaoui, Zairi, et al., 2013; Bergaoui, Zairi, et al., 2013; Mechlia et al., 2019). Recently, derivatives of dermaseptin have also been tested on coronavirus family computationally and show the strongest antiviral activity (Mustafa et al., 2019). Given these results, the affinity of several dermaseptin derivatives for SARS-CoV-2 spike protein through *in silico* was demonstrated.

METHODS

Preparation of the SARS-CoV-2 Spike Protein Macromolecule

In this study, the macromolecule used was the spike protein of SARS-CoV-2 obtained from Protein Data Bank (http://www.rcsb.org/pdb) with PDB ID 6LZG (Wang et al., 2020). The macromolecule was then prepared by removing water molecules, adding polar hydrogen atoms, and calculating Kollman's partial charge.

Preparation of the Antiviral Peptide Molecules

In this study, the molecules used are dermaseptin-S4 and dermaseptin-S9 antiviral peptide sequences which were produced by frogs of the genus *Phyllomedusa* and have been sequenced using PEPFOLD 3.5 (http://bioserv.rpbs.univ-paris-diderot.fr/PEP-FOLD/) (Figure 1). PEP-FOLD 3.5 is a server used to model peptide sequences with amino acid counts between 5 and 50 into three-dimensional conformation using the *de novo* method (Lamiable et al., 2016; Maupetit et al., 2009; Shen et al., 2014; Thévenet et al., 2012). The results of the peptide molecular modeling were then used as input for protein-peptide docking simulations.

The Simulations of Protein-Peptide Docking

In this study, HPEPDOCK was used to simulate protein-peptide docking (Huang & Zou, 2007, 2008; Yan et al., 2017; Zhou, Jin, et al., 2018; Zhou, Li, et al., 2018). The two antiviral peptide molecules for docking simulations were modeled and polar hydrogen atoms were added using the *de novo* method. Then the protein-peptide complex type was selected by RMSD 4.0 Å grouping. The shape of the Connolly point surface of the molecule into different components including convex, concave, and flat patch was produced through the HPEPDOCK algorithm. HPEPDOCK optimized, refined, overhauled, and re-selected the side chain interface from the top 10 candidate solutions. It also changed the relative orientation of the molecule by limiting flexibility in the side chains of the interacting surface and allowing the movement of small and rigid objects. Analysis of the results of protein-peptide docking simulations was performed using Discovery Studio 2020 (Sharma, 2019; Sharma et al., 2019).

Preparation of ACE-2 Receptor Macromolecule

In this study, ACE-2 receptor macromolecule was downloaded from Protein Data Bank (http://www.rcsb.org/pdb) with PDB ID 2AJF (Li et al., 2005). This receptor macromolecule was prepared by removing water molecules, adding polar hydrogen atoms, and calculating Kollman's partial charge.

The Simulations of Protein-Protein Docking

In this study, pyDockWEB was also used to simulate docking of protein-protein between the two peptide complexes resulting from protein-peptide docking to ACE-2 receptor (Ahuja & Singh, 2016; Sable & Jois, 2015; Jiménez-García et al., 2013). The RMSD 4.0 Å grouping was used and the protein-protein complex type was selected. The surface representation of the Connolly point from the molecule into different components such as convex, concave, and flat patch was generated through the pyDockWEB algorithm. pyDockWEB optimized, refined, overhauled, and re-selected the side chain interface from the top 10 candidate solutions. It also changed the relative orientation of the molecule by limiting flexibility in the side chains of the interacting surface and allowing the movement of small and rigid objects. The suitability of the system was verified by visualization observations using Discovery Studio 2020 (Sharma, 2019; Sharma et al., 2019).

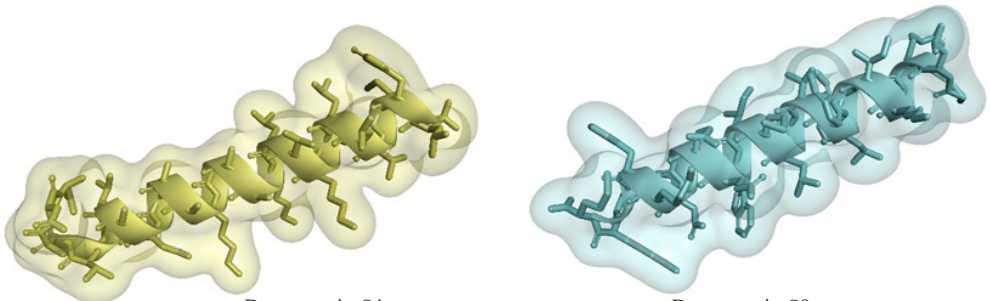

Figure 1. The molecular structure of the antiviral peptides used in this study

RESULTS AND DISCUSSION

The Simulations of Protein-Peptide Docking

The two antiviral peptides produced by frogs of the genus *Phyllomedusa* that have been modeled with PEP-FOLD 3.5 server were then identified and evaluated for their affinity and interaction with the spike protein macromolecule of SARS-CoV-2 that has been prepared through protein-peptide docking simulations using the HPEPDOCK algorithm. The results of protein-peptide docking simulations in Table 1 show that dermaseptin-S9 peptide has the best affinity with the active binding site of SARS-CoV-2 spike protein compared to dermaseptin-S4 peptide, with binding free energy values of −792.93 kJ/mol and −692.43 kJ/mol, respectively. This phenomenon proves that there is a promising sign of dermaseptin-S9 peptide as an inhibitor of SARS-CoV-2 spike protein because it has strong binding and interactions in the active site area of the target macromolecule. Through protein-protein docking simulations, the ability of the peptide-protein complex that has been formed in inhibiting its attachment to the ACE-2 (Angiotensin Converting Enzyme-2) receptor macromolecule was further demonstrated.

Table 1. The affinity of the antiviral peptide molecules against the SARS-CoV-2 spike protein macromolecule

Peptide molecule	Binding free energy (kJ/mol)
Dermaseptin-S4	−692.43
Dermaseptin-S9	−792.93

Based on the visualization of protein-peptide docking simulation results, dermaseptin-S4 peptide and dermaseptin-S9 peptide show different conformational molecular interactions with the active site of SARS-CoV-2 spike protein. However, the two peptide molecules can occupy the polar patch in the active binding site of SARS-CoV-2 spike protein macromolecule (Figure 2). This phenomenon can occur because the HPEPDOCK algorithm allows peptide molecules to move freely without rigid bonds to the molecular docking system used. Overall, interactions formed by dermaseptin-S9 peptide were more numerous than dermaseptin-S4 peptide, with eighteen interactions including nine hydrogen bonds (with Asn370, Ser375, Thr376, Lys378, Gly404, Asp405, and Gln414), eight hydrophobic interactions (with Tyr369, Ala372, Pro384, Lys386, and Arg408), and one electrostatic interaction (with Arg408).

On the other hand, dermaseptin-S4 peptide only forms seven interactions consisting of six hydrogen bonds (with Leu335, Pro337, Phe338, Asn343, Val367, and Ser373) and one hydrophobic interaction (with Phe338) (Table 2). In general, dermaseptin-S4 and dermaseptin-S9 peptide molecules have different interactions with the active site area of SARS-CoV-2 spike protein. However, the binding site of dermaseptin-S9 peptide molecule is predicted to be more potent as a location for attaching inhibitory molecules suitable for the SARS-CoV-2 protein spike macromolecule, especially compounds with large molecular weights. This is due to amino acid residues in the area more than in the binding location of

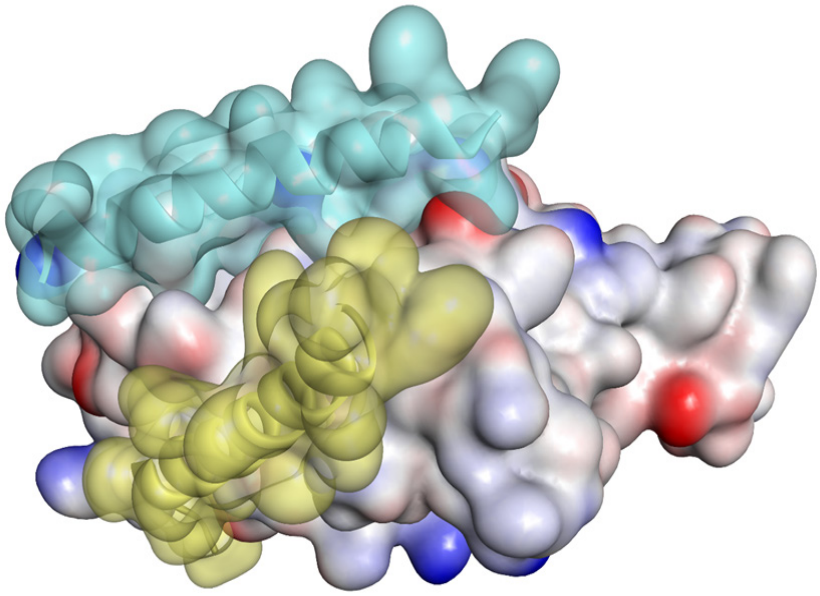

Figure 2. The poses of Dermaseptin-S4 peptide (yellow) and Dermaseptin-S9 peptide (blue) in the binding site area of SARS-CoV-2 spike protein macromolecule

Table 2. The interactions formed between antiviral peptide molecules and SARS-CoV-2 spike protein macromolecule

Peptide molecule	Number of interactions	Amino acid residues
Dermaseptin-S4	7	Leu335, Pro337, Phe338, Asn343, Val367, Ser373
Dermaseptin-S9	18	Tyr369, Asn370, Ala372, Ser375, Thr376, Lys378, Pro384, Lys386, Gly404, Asp405, Arg408, Gln414

the dermaseptin-S4 peptide molecule. This phenomenon is proven by the ability of the dermaseptin-S9 peptide molecule to form strong and stable interactions with the target macromolecules.

The dominant interaction of the peptide-protein complex is hydrogen bonds, especially peptide molecules which act as hydrogen bonds donors and amino acid residues in the target macromolecule as hydrogen bond acceptors. Most hydrogen bonds between peptide molecules and protein macromolecules are relatively strong, with average bond lengths starting at 3 Å. In addition to hydrogen bonds, there are also contributions from hydrophobic interactions and interestingly there are electrostatic interactions in dermaseptin-S9 peptide complex. It can be predicted that hydrogen bonds, hydrophobic interactions, and electrostatic interactions play an important role in stabilizing peptide-protein complexes.

The Simulations of Protein-Protein Docking

Thereafter, to ensure the ability of each peptide-protein complex to prevent the attachment of SARS-CoV-2 spike protein macromolecule to the surface of ACE-2 receptor, identification, evaluation, and exploration were carried out using the protein-protein docking methods. The best affinity with strong and stable molecular interactions of the peptide-protein complex is predicted to prevent the entry of SARS-CoV-2 into cells and host tissues due to their inability to reach ACE-2 receptor in the process of SARS-CoV-2 infection signaling. It is also important to observe amino acid residues that play an important role in inhibiting the formation of molecular interactions between the binding site of SARS-CoV-2 spike protein macromolecule and the surface area of ACE-2 receptor.

Based on the results of the protein-protein docking simulations in Table 3, dermaseptin-S4 peptide complex and dermaseptin-S9 peptide complex have positive total energy, with values of 303.87 kJ/mol and 517.85 kJ/mol, respectively. Interestingly, dermaseptin-S9 peptide complex has a better total energy value than dermaseptin-S9 peptide complex due to the contribution of electrostatic interactions and van der Waals interactions that exist between the protein-protein complexes formed. This phenomenon shows that both dermaseptin peptide molecules bound to SARS-CoV-2 spike protein macromolecule can prevent the attachment of ACE-2 receptor so that the infection signal can be prevented. The interaction between peptide-protein complexes in both dermaseptin peptide molecules is also dominated by unfavorable interactions. Given this interaction, it is thought that the formation of infection signals in SARS-CoV-2 will not occur.

CONCLUSION

The dermaseptin-S9 peptide molecule can form a stable and strong molecular interaction with the active site of the binding of SARS-CoV-2 spike protein macromolecule. This is indicated by the best affinity value compared to dermaseptin-S4 peptide molecule, with a free binding energy value of −792.93 kJ/mol. Dermaseptin-S9 peptide molecule is also able to prevent the attachment of SARS-CoV-2 spike protein macromolecule to the surface of ACE-2 receptor because it has positive total energy, with a value of 517.85 kJ/mol. Based on the results of this study it was proven that dermaseptin-S9 peptide molecule has the potential as a candidate for SARS-CoV-2 spike protein macromolecule inhibitor in controlling COVID-19 disease.

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Table 3. The total energy of the peptide-protein complexes against ACE-2 receptor macromolecule

Peptide-protein complex	Electrostatics (kJ/mol)	Desolvation (kJ/mol)	van der Waals (kJ/mol)	Total energy (kJ/mol)
Dermaseptin-S4 + SARS-CoV-2 Spike Protein	183.17	42.74	77.96	303.87
Dermaseptin-S9 + SARS-CoV-2 Spike Protein	176.69	85.20	255.96	517.85

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