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Comparative analysis of the stability features of human alpha-defensins as candidates for the future COVID-19 therapy through molecular dynamics

Taufik Muhammad Fakih[1*], Dwi Syah Fitra Ramadhan[2], Arfan[3]
[1]Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Islam Bandung
Jl. Ranggagading No.8, Tamansari, Kec. Bandung Wetan, Bandung, West Java, Indonesia
[2]Department of Pharmacy, Poltekkes Kemenkes Makassar,
Jl. Baji Gau No.10, Baji Mappakasunggu, Kec. Mamajang, Makassar, Sulawesi Selatan, Indonesia
[3]Faculty of Pharmacy, Universitas Halu Oleo,
Jl. H.E.A Mokodompit, Anduonohu, Kec. Kambu, Kota Kendari, Sulawesi Tenggara, Indonesia

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ABSTRACT

Coronavirus 19 (COVID-19) is still a global health issue to date, SARS-CoV-2 is a novel coronavirus that is responsible for this sickness. The receptor-binding domain of the SARS-CoV-2 virus associates with angiotensin-converting enzyme 2 (ACE-2) and allows the virus to enter human cells. Natural peptides such alpha-defensin are thought to attach to the SARS-CoV-2 RBD and prohibit it from engaging with ACE-2. Molecular dynamics simulations using a computational approach are utilized to understand the stability of six alpha-defensin macromolecules using the Gromacs 2016 software. The trajectories formed are then analyzed using VMD 1.9.4 and BIOVIA Discovery Studio 2020 software. Finally, the free energy is estimated using the MM/PBSA method. The alpha-defensins 2 macromolecules were found to have the best stability based on numerous study results (trajectory visualization, RMSD, RMSF, and free energy calculations). As a result, these macromolecules could be used to build new antiviral treatments for COVID-19 infectious disease candidates.

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Keywords: COVID-19, infectious disease, SARS-CoV-2 RBD, alpha-defensin, molecular dynamics, computational approach

Corresponding author:
Taufik Muhammad Fakih
Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Islam Bandung
Jl. Rangga Gading No. 8, Tamansari, Bandung, West Java, Indonesia
Email: taufikmuhammadf@gmail.com

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is triggered by the new coronavirus identified as SARS-CoV-2, which is a rising worldwide health concern (Zhou et al., 2020). According to the WHO situation assessment on COVID-19, 46 million cases have been reported worldwide as of November 1, 2020 (WHO, 2020). Based on these numbers, the United States had the highest number of cases (9.2 million), followed by Australia, Belgium, Cambodia, Canada, China, France, Finland, Germany, India, Italy, Japan, Malaysia, Nepal, Philippines, Republic of Korea, Russian Federation, Singapore, Spain, Sri Lanka, Sweden, United Arab Emirates, United Kingdom, Thailand, and Vietnam. So far the COVID-19 disease has claimed 1.2 million victims, with a death rate of around 2.6% (Battegay et al., 2020; Chen et al., 2020).

The virus enters human cells via attaching to the spike protein's receptor-binding domain, particularly interfaces to angiotensin-converting enzyme 2 (ACE-2) (Hoffmann et al., 2020; Walls et al., 2020; Chen Wang et al., 2020; Wu et al., 2020). The protein ACE-2, a homolog of the renin-angiotensin system (RAS) enzyme angiotensin-converting enzyme (ACE), is expressed in many human organs and tissues. It has a wide range of biological functions and can help to mitigate the negative effects of the RAS in a number of illnesses (Holly et al., 2017; Wilson et al., 2013). Because 2019-nCoV shares 79.5 percent of the total genomic sequence identity with SARS-CoV, the pathogenic mechanism of COVID-19 may be comparable to that of SARS (Fakih & Dewi, 2020). Although COVID-19 mediated by SARS-CoV-2 has a lower overall risk of mortality than SARS and MERS, severe symptoms typically encounter organ dysfunction, involving acute respiratory distress syndrome (ARDS), renal insufficiency failure, adverse cardiac injury, and severe hepatic injury (Ni et al., 2020).

Interfering with the binding of the SARS-CoV-2 receptor-binding domain (RDB) to ACE-2 using a designed medication offers the potential to prevent the virus from entering human cells while also presenting novel therapeutic options. Alpha-defensins are influential inherent immune system transcription factors comprising antiviral, antifungal, and antibacterial activities, according to earlier research (Bonanzinga et al., 2017; Deirmengian et al., 2015). Human alpha-defensins have been shown to have potent antiviral effect against SARS-CoV-2 pseudotype viruses in previous research (Xu et al., 2021). Alpha-defensins can bind to both ACE2 and the SARS-CoV-2 spike protein at the same time (Gao & Zhu, 2021). In cell culture, defensin exerts direct antiviral activity through distinct pathways for different viruses. Furthermore, defensins can modulate the natural and adaptive immune responses towards viral infections according to their considerable immunomodulatory capabilities. Several earlier investigations have demonstrated that computational tools can be used to estimate or even generate potential COVID-19 therapy medications (Zhang et al., 2020).

This work will use computational molecular dynamics simulations to predict the stability of alpha-defensins as a potential binder with SARS-CoV-2-RBD that can impede its interaction with ACE-2. A computational approach is a new field of research that draws on breakthroughs in molecular and structural biology, immunology, and bioinformatics (Ibrahim et al., 2018). Specifically in materials science, food chemistry, and pharmaceuticals, the structural and interaction components of biomaterials can benefit from the strong foundation that molecular dynamics modeling methods have reached for investigating the thermodynamic, dynamic, and interactive properties of biomolecules (Chun et al., 2018). Hence, the outcomes of this research can serve as a starting point for any further investigation into alpha-defensin action through in vitro and in vivo approaches.

METHODS

The preparation of alpha-defensin macromolecules

Six varieties of alpha-defensins were employed in this study, and they were retrieved from the Protein Data Bank (http://www.rcsb.org/pdb) with their PDB IDs 3GNY (alpha-defensin 1) (Wei et al., 2009), 1ZMK (alpha-defensin 2) (Xie et al., 2005), 1DFN (alpha-defensin 3) (Hill et al., 1991), 1ZMM (alpha-defensin 4), 1ZMP (alpha-defensin 5), and 1ZMQ (alpha-defensin 6) (Szyk et al., 2006). Downloaded alpha-defensin macromolecules were then processed in advance by water and ligand

molecules are removed, polar hydrogen atoms are added, and Kollman's charge is calculated using AutoDock 4.2 with MGLTools 1.5.6 (Huey et al., 2012).

The simulation of molecular dynamics

Gromacs 2016 was used for simulations, while VMD 1.9.4 and BIOVIA Discovery Studio 2020 were used for analyses (Kutzner et al., 2019; Vermaas et al., 2016). To parameterize alpha-defensin macromolecules, AMBER99SB-ILDN and AMBER general force field (GAFF) were utilized (Smith et al., 2015). The Ewald Particle Mesh method was used calculate the electrostatic charge beyond a radius (Isele-Holder et al., 2012). Mineralization of the system was achieved by introducing Na + and Cl- ions. To solve complex systems, the TIP3P cubic water model was employed. Minimization, starting to heat up to 310 K, temperature balancing (NVT), pressure complement (NPT), and a production run with a time step of 2 fs for 100 ns are all included in the simulation stage. Energy, temperature, pressure, root mean square deviation (RMSD), and root mean square fluctuation were all used to verify system stability (RMSF). During molecular dynamics simulations, the RMSD and RMSF values of macromolecules were calculated to determine the alpha-defensin macromolecular stability.

The analysis of trajectory visualization

The trajectory of the molecular dynamics simulation results was evaluated using VMD 1.9.4 and BIOVIA Discovery Studio 2020 software in order to provide information in the form of molecular dynamics animation to observe the properties and characteristics of alpha-defensin macromolecules (Van den Berge et al., 2020).

The identification of root mean square deviation (RMSD)

Root Mean Square Deviation (RMSD) is a technique for analyzing discrepancies in macromolecular structures before and after molecular dynamics simulations. This analysis was performed on trajectories to observe conformational changes that occur during the simulation. The analysis method for RMSD can show a comparison between the structure of the alpha-defensin macromolecule which is folded, partially open, and completely open. This phenomenon indicates a dynamic change associated with the modification of alpha-defensin macromolecules (Martínez, 2015).

The evaluation of root mean square fluctuation (RMSF)

The displacement of a set of atoms in comparison to a reference structure or the average structure of a molecular dynamics simulation is measured by Root Mean Square Fluctuation (RMSF). RMSF calculations were accomplished on the trajectory of the simulation results to identify the flexible portion in alpha-defensin macromolecules and residues that have low RMSF values are considered to have better stability (Islam et al., 2020).

The calculation of MM/PBSA free energy

The g_mmpbsa package, which is included in Gromacs 2016, was used to show the calculation of free energy from molecular dynamics simulation using the MM/PBSA approach (Ren et al., 2020). The desolvation of the energetic poles was determined by calculating using the Poisson-Boltzmann equation with a 0.5 grid size. To represent water as a solvent, the dielectric constant of the solvent is set to 80 (Kumari et al., 2014). Measuring the contact area that a solvent with a 1.4 solvent radial distance might penetrate was used to determine the nonpolar contribution. The free energy of alpha-defensin macromolecules was calculated using 200 snapshots recorded from the start of the molecular dynamics simulation to the finish.

RESULT AND DISCUSSION

Trajectory visualization analysis

This work used optimization to determine the stability of the alpha-defensin macromolecular structure using computational molecular dynamics simulations. In the molecular dynamics simulation

stage, the six macromolecules were chosen for preparation. This simulation can provide further explanation regarding structural information, dynamics, and energy stability. Macromolecular structures were made not rigid so that they can move according to their flexibility and move not only limited to rotation, but also translation. The trajectory is a snapshot of the alpha-defensin macromolecular system that represents atomic coordinates across time as a consequence of molecular dynamics simulations. This trajectory data can explain information such as simulation visualization to observe the characteristics of a system during the simulation (Chakraborty & Zheng, 2015).

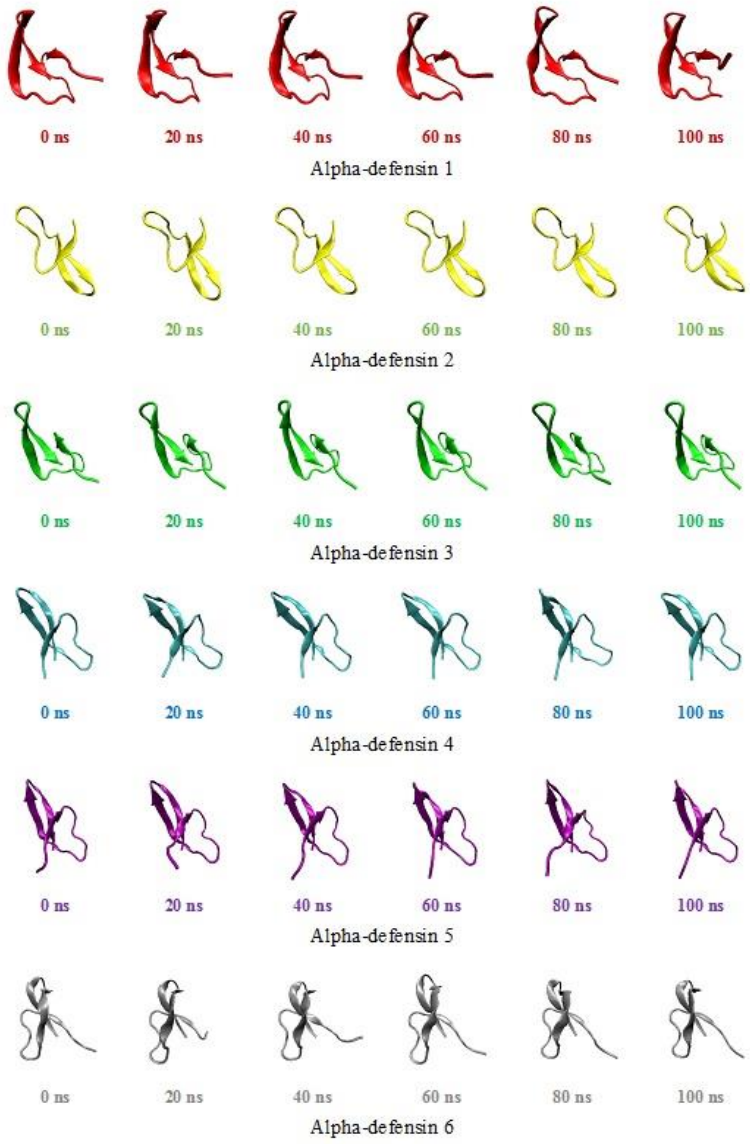

Figure 1. Molecular dynamics simulations of alpha-defensin macromolecular conformational snapshots

Based on snapshots obtained every 20 ns from molecular dynamics simulation results, it can be seen that each alpha-defensin macromolecule has no substantial conformational change in general (Figure 1). Overall, the alpha-defensin macromolecule poses vary during the simulation, but changes only occur in the loop part of the macromolecule. Meanwhile, the other parts are still stable in the form

of alpha-helix and beta-sheet. This event is expected to improve alpha-defensin macromolecules' ability to interact with the active site of target receptor macromolecules, particularly in SARS-CoV-2-RBD (Wang et al., 2020; Zhao et al., 2016).

RMSD and RMSF graph identification

To ensure the stability and logic of the chosen conformation, the RMSD value of the alpha-defensin macromolecule was calculated. The simulation outcomes trajectory is also subjected to this analysis in order to identify the equilibration period and quality of the biomolecular simulation, as well as to assess the conformational changes that occur during the simulation (Khezri et al., 2018). The graph in Figure 2 shows that the six systems can maintain a stable conformation until the end of the simulation. However, when compared to other alpha-defensin macromolecules, alpha-defensins 2 macromolecules have the best stability, with an average RMSD value of 1.47 Å. According to the simulation results, from 20 ns to the end of the molecular dynamics simulation, all macromolecules begin to reach stability. Similar to the visualization of the snapshot trajectory, the six macromolecules have constant stability. These results were predicted by average RMSD values of alpha-defensins macromolecules ranging from only 1.4 Å to 1.9 Å.

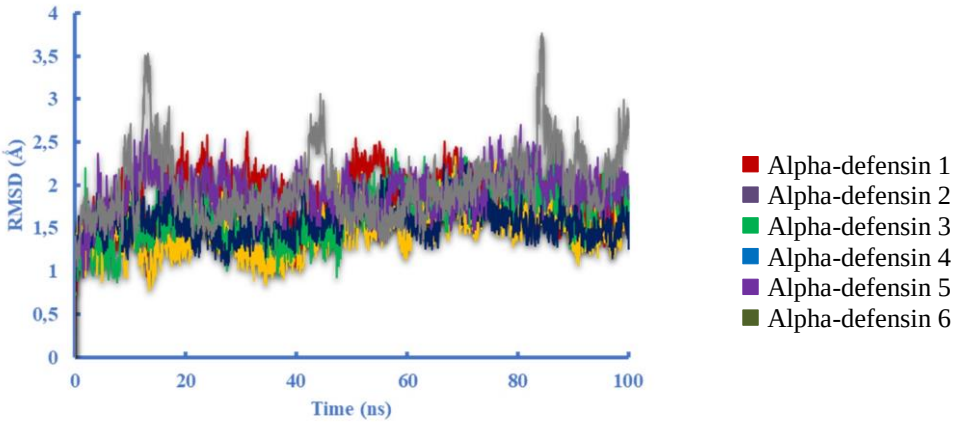

Figure 2. Graph comparison of the root mean square deviation (RMSD) of alpha-defensins macromolecules

Throughout the simulation, the alpha-defensins 6 macromolecule was the system that fluctuated the most, especially at 10 ns, 40 ns, and 80 ns until 100 ns. This phenomenon indicates that there are several significant conformational changes of these macromolecules. Nevertheless, further observations using RMSF analysis are needed to evaluate conformational changes to the initial structure. The RMSF calculation is used to identify the flexible part of a system, amino acid residues having a low average RMSF value are considered to have good stability during molecular dynamics simulations (Khan et al., 2020).

During the molecular dynamics simulation, Figure 3 displays the flexibility of the amino acid residues that make up the alpha-defensins macromolecule. Most of the residues had high flexibility, except for the alpha-defensin 1, 2, and 4 macromolecules which had average RMSF values of 1.06 Å, 1.08 Å, and 1.05 Å, respectively. Meanwhile, other macromolecules had a fairly high average RMSF value, especially alpha-defensin 6 macromolecules, with an average RMSF value of 1.31 Å. Because the amino acid residues at the terminals of the alpha-defensin macromolecule play such a crucial role in binding to the target receptor, they have a lot of flexibility (Tai et al., 2020; Tian et al., 2020).

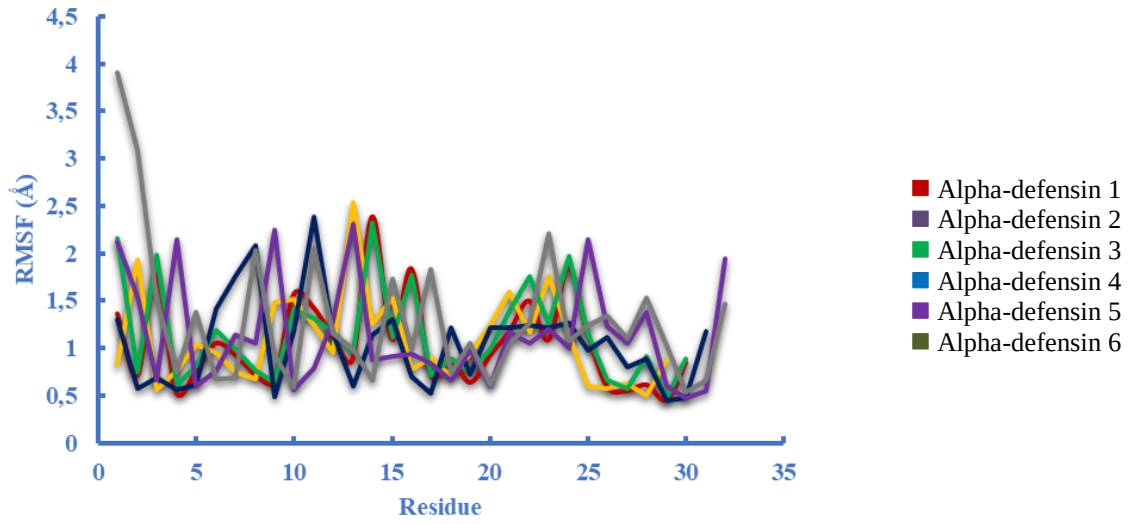

Figure 3. Graph comparison of the root mean square fluctuation (RMSF) of alpha-defensins macromolecules

MM/PBSA free energy calculation

During molecular dynamics simulations, the MM/PBSA method is utilized to calculate the value of free energy. Free energy is the sum of numerous calculations, including gas-phase potential energy, polar and non-polar free energy, and entropy, which can be determined directly from simulation results using several conformations. Because of its high accuracy, the MM/PBSA method is commonly employed in the estimation of free energy (Swanson et al., 2004). According to MM/PBSA calculations, alpha-defensins 2 macromolecules have a lower free energy value than other macromolecules, with a value of −1687.14 kJ/mol (Table 1).

Table 1. Free energy of alpha-defensisns macromolecules during molecular dynamics simulations

Macromolecule	Free Energy (kJ/mol)
Alpha-defensin 1	−1144.04
Alpha-defensin 2	−1687.14
Alpha-defensin 3	−1261.99
Alpha-defensin 4	−1216.04
Alpha-defensin 5	−1271.99

Alpha-defensins 6 has the lowest free energy value during molecular dynamics simulations, with a free energy value of −1031.05 kJ/mol, in contrast to alpha-defensin 2 macromolecules. The more negative the free energy value indicates a good level of stability, so the bonds formed will be stronger (Darusman & Fakih, 2020). This result correlates with the low stability of these macromolecules which have been identified based on both RMSD and RMSF graphs. Finally, the macromolecular structure of alpha-defensin 2 could be a promising candidate in the development of COVID-19 treatments that target SARS-CoV-2-RBD.

CONCLUSION

The data obtained from the research results show that alpha-defensins 2 has the best stability during molecular dynamics simulations. The average RMSD value of these macromolecules was 1.47 Å, while the average RMSF value was in the range of 1.08 Å. Interestingly, with a free energy value of−1687.14 kJ/mol, this macromolecule likewise possesses a good free energy. Therefore, alpha-

defensins 2 macromolecules can serve as a major factor in protecting lung tissue from COVID-19 viral infection.

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