Molecular Docking Simulation of Neuraminidase Influenza A Subtype H1N1 with Potential Inhibitor of Disulfide Cyclic Peptide (DNY, NNY, LRL)

R P Putra¹, R Imaniastuti¹, M A F Nasution¹, Djati Kerami², U S F Tambunan¹,a)

¹Bioinformatics Research Group, Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Indonesia, Universitas Indonesia, Depok 16424 Indonesia
²Mathematics Computational Group, Department of Mathematics, Faculty of Mathematics and Natural Science, Universitas Indonesia, Depok 16424 Indonesia

Email: usman@ui.ac.id

Abstract: Oseltamivir resistance as an inhibitor of neuraminidase influenza A virus subtype H1N1 has been reported lately. Therefore, to solve this problem, several kinds of research has been conducted to design and discover disulfide cyclic peptide ligands through molecular docking method, to find the potential inhibitors for neuraminidase H1N1 which then can disturb the virus replication. This research was studied and evaluated the interaction of ligands toward enzyme using molecular docking simulation, which was performed on three disulfide cyclic peptide inhibitors (DNY, LRL, and NNT), along with oseltamivir and zanamivir as the standard ligands using MOE 2008.10 software. The docking simulation shows that all disulfide cyclic peptide ligands have lower Gibbs free binding energies (∆Gbinding) than the standard ligands, with DNY ligand has the lowest ∆Gbinding at -7.8544 kcal/mol. Furthermore, these ligands were also had better molecular interactions with neuraminidase than the standards, owing by the hydrogen bonds that were formed during the docking simulation. In the end, we concluded that DNY, LRL and NNT ligands have the potential to be developed as the inhibitor of neuraminidase H1N1.

1 Introduction

Influenza is a severe infectious disease caused by a virus and may infect humans and animals. In general, the common symptoms of this disease are fever, sore throat, headache, and cough. In more severe cases, it may lead to death for someone who infected by several dangerous types of influenza virus [1]. In 2009, influenza A subtype H1N1 virus became a pandemic, with a new viral strain identified in April 2009 and widely known as swine flu. This pandemic caused a fatal infection with more than 80 deaths in 40 countries, first detected in Mexico and spreading to various countries in South and North America, Europe and Asia [2]. One method for the prevention of H1N1 virus infection that has been suggested earlier is by vaccination. However, the current vaccine is thought to still have weakness and imperfections in protecting the body when an influenza virus occurs. This is caused by the mutation of the virus. This mutation may be due to antigenic drift or antigenic shift [3]. Based on its antigenic properties, influenza viruses have two functional surface glycoproteins namely hemagglutinin and neuraminidase (NA). The H1N1 virus is a virus subtype belonging to the type of influenza A virus. This kind of virus consists of sixteen hemagglutinins (H1-H16) and nine neuraminidases (N1-N9).
Theoretically, there are 144 subtypes, and until now 105 subtypes of influenza A virus have been found, and all are endemic to aquatic birds. However, some subtypes have been found in chickens and mammals [4]. At this time, various types of antivirals have been developed as a substitute for the vaccine method. Oseltamivir and zanamivir, which can block the development of H1N1 virus by inhibiting the NA, have been proposed and recommended the Centers for Disease Control and Prevention (CDC) and World Health Organization (WHO) to treat and cure the influenza flu patient[5]. Moreover, Oseltamivir is one of the antiviral drugs capable of inhibiting NA that functions in the viral replication process at the last stage of viral life cycle. The interaction between oseltamivir and NA is found in its interaction with the catalytic residue of the NA active site when the residual framework stabilizes the enzyme structure. However, it has also been reported that the oseltamivir resistance to influenza virus has increased [6]. Hence, the development of new antiviral to substitute oseltamivir as the anti-influenza agents is urgently needed.

In this study, the computational study of influenza A subtype H1N1 was conducted using the molecular docking methods [7]. The purpose of this study was to examine the interaction and conformation of three potential disulfide cyclic peptide inhibitors (DNY, NNY, LRL) with NA of influenza virus subtype H1N1 through molecular docking simulation methods. Also, this study also evaluates and determines potential ligands to be developed as a drug.

2 Materials and Methods

2.1 Sequence searching and preparation of the Influenza A virus (H1N1) neuraminidase

The NA sequence of influenza A virus (H1N1) used in this study can be downloaded from an existing database at NCBI via the site http://www.ncbi.nlm.nih.gov/genomes/flu/ by using a computer device connected to the internet. The NA sequence used has a GenBank code ACR08499.1. The homology modeling process for the three-dimensional (3D) structure of H1N1 NA can be done by using SWISS-Model which can be accessed via http://swissmodel.expasy.org/SWISS-MODEL.html [8–10]. After which, the 3D structure of H1N1 NA then downloaded with the PDB file format.

Visualization of the catalytic side of H1N1 NA was performed with MOE 2008.10 software through ‘Site Finder’ and ‘Map’ features. Furthermore, the process of geometry optimization and energy minimization of three-dimensional NA structures were carried out using the same software as well. The first step is protonation with ‘Protonate 3D’ feature to add the hydrogen atoms into the enzyme structure. Then, the MMFF94x force field was applied as the partial charge and optimization. When optimizing, the enzyme was solvated in ‘Gas Phase’ and RMS gradient of 0.05 kcal/mol.Å was applied. Other parameters were defined using the default protocols, and finally, the output files were saved in .moe file format.

2.2 Designing the 2D structure of the cyclic peptide ligands

The amino acid sequences of cyclic peptides that have been designed are then modeled into three-dimensional structures. This modeling step was done using ACD-Labs software, which then the results were stored in MDL Molfile (.mol) format. After which, the files were converted and optimized using VegaZZ software. Finally, the ligand database was made in MOE 2008.10 software by including all cyclic peptide design into MOE database viewer in .mdb file format. The optimization process begins by performing ‘Wash’ protocol for all cyclic peptides. Then, the partial charge of the ligands was loaded using ‘Partial Charge’ feature with the MMFF94 (modified) force field was applied to all ligands. Then, the ‘Gas Phase’ method was applied to the solvation mode of all ligands with RMS gradient 0.001 kcal/Å.mol. Finally, the output file was later saved in .mdb format.
2.3 Molecular Docking Simulation

The molecular docking simulation process was performed with the MOE 2008.10 through MOE \rightarrow Simulation \rightarrow Dock. The placement method of ‘Triangle Matcher’ was used with a repetition of energy readings per 1,000,000 positions and other parameters present in MOE. Moreover, ‘London dG’ was used as the Rescoring 1 function with the repetition population of 1000. The first repetition counted 30 times and the second set shows only one best result of 100 repetitions. After which, the Gibbs free binding energy ($\Delta G_{\text{binding}}$) value, hydrogen bond interactions and ligand’s residues contact were observed and analyzed to determine the best ligand among all.

3 Results and Discussions

3.1 Sequence searching and preparation of the Influenza A virus (H1N1) neuraminidase

The sequence of NA data used as an enzyme target was determined using a database that presented on the NCBI website. This step was performed using the same parameters as we are done in previous work [11]. The NA sequence used was the Auckland NA sequence in 2009, NA [A/Auckland/1/2009 (H1N1)] with the genogram code of ACR08499.1. In this study, the resulting 3D NA structure has a 99.479% identical identity percentage of the template used (PDB ID: 3NSS). This significant percentage of identity value indicates that this 3D NA structure is good enough for use in later stages of research.

Determination of the catalytic site of NA was performed based on a study of viral sequences, followed by the literature study from NCBI sequence search results. From these results, it is known that NA has a catalytic side consisting of seven functional amino acids, namely Arg118, Asp151, Glu278, Arg293, Arg368, Tyr402, and Glu425. From the visualization results (Figure 1), it appears that the catalytic site residue is located on the surface of the enzyme and forms a large cavity.

Figure 1. The visualization of catalytic site of Influenza A (H1N1) neuraminidase

NA optimization process needs to be done before molecular docking simulation with enzyme objective being in optimum condition when the simulation is done. In this study, the first step of the optimization process was done with ‘Protonate 3D’ program in MOE 2008.10 software, it is aimed to enzyme turn into the protonated state, followed by the addition of partial charge. Finally, the energy minimization of the enzyme was performed to avoid unrelated van der Waals contacts and to minimize high-energy steric effects of the structure [12]. The parameter setting of energy minimization was done in the optimal
settings, with MMFF94x, RMS gradient of 0.05 kcal/molÅ and gas phase solvation were used as the main parameters [13,14]. Later, the output file was saved in .moe format.

3.2 Designing the 2D structure of the cyclic peptide ligands

The design of amino acid peptide sequence was done by using ACD/ChemSketch software. The peptides, then called ligands, are the cyclic disulfide peptide generated from the previous studies, namely DNY, NNY, and LRL ligands [11]. In addition to these three ligands, zanamivir and oseltamivir which act as the standard ligands were designed as well. The ligands DNY, NNY and LRL are cyclic disulfide pentapeptide form comprising two amino cysteine acids with disulfide bonds, and three other amino acids, as it displayed in Figure 2. The cyclization form of amino acid itself was aimed to improve the stability of peptide molecules when introduced into the body, so proteases do not easily truncate them.

![Figure 2. The scheme of the structured cyclic peptide designed in this research](image)

The peptide and standard (oseltamivir & zanamivir) ligands already in the .mol format were imported into the MOE 2008.10 database viewer for optimization and minimization. The first step was washing for the entire ligand in order to improve the position of the hydrogen atoms found in the ligand to improve the ligand structure. Then, the partial charge was performed, followed by the energy minimization process with RMS gradient 0.001 kcal/mol Å [15].

3.3 Molecular docking simulation result and analysis

Molecular docking is a computational method to detect the exact bonding bond between the ligand and the receptor [16]. In this research, we used MOE 2008.10 as the primary tool for docking simulation purpose [17]. Docking process was performed on three potential peptide ligands (DNY, NNY, LRL) as well as the standard ligands (zanamivir and oseltamivir) against the catalytic side of NA [11]. In the process of docking, the enzyme is in rigid condition while the ligand is in a flexible condition so that it can be free to move and rotate. Based on the docking simulation results, it was found that the DNY ligand has the most negative ΔGbinding value and shows that DNY ligand has the strongest bond to the enzyme compared to the other two peptide enzymes (NNY and LRL) as well as the standard. The following results are displayed in Table 1.
Table 1. The result of molecular docking simulation and molecular interaction between the NA binding site and the ligands, red fonts indicate that this residue is the catalytic site of the NA

| No. | Ligand Name | ΔG_{binding} (kcal/mol) | pKi  | Interacted Amino Acid Residues         |
|-----|-------------|-------------------------|------|---------------------------------------|
| 1   | DNY         | -7.8544                 | 5.7243 | Glu119, Ile149, Asp151, Asp151, Arg118, Arg152, Ser247, Arg293, Asn344, Arg368, Arg368, Tyr402 |
| 2   | NNY         | -6.8994                 | 5.0283 | Glu278, Asn344, Gly345, Arg118, Arg156, Arg368, Tyr402 |
| 3   | LRL         | -6.8056                 | 4.9599 | Ile149, Asp151, Trp179, Trp179, Glu228, Tyr402, Ser247, Arg368, Arg368 |
| S1  | Zanamivir   | -6.5858                 | 4.7997 | Glu119, Glu119, Ser247, Glu277, Glu278, Ser247, Arg293, Arg368, Arg368 |
| S2  | Oseltamivir | -4.1582                 | 3.0305 | Glu119, Glu278, Tyr402, Arg156 |

Docking process analysis is not only seen from ΔG_{binding} value, but also from the interaction between ligand with enzyme through residue contact especially hydrogen bond. The hydrogen bonds that occur in the ligand-enzyme complex can be identified and analyzed in the ligand interaction program using the MOE 2008 software as well. From the Table 1, DNY, LRL, and NNY ligands have an interaction with thirteen, seven, and nine amino acid residues in the catalytic site of NA, respectively. With the DNY, LRL, and NNY ligands forming six, four, and three hydrogen bonds. Additionally, the zanamivir and oseltamivir itself interact with nine and four amino acid residues, respectively, followed by 4 and 2 hydrogen bonds that created with the former and latter standard ligands. From these results, it can be analyzed that peptide ligands have better interaction and inhibition activity of NA enzyme compared to the standard ligands.

4 Conclusions
In this study, the DNY, LRL, and NNY ligands were deployed to know their inhibition activity and interaction in the catalytic site of the NA enzyme. Docking studies show that all three peptide ligands have better inhibition activities than both oseltamivir and zanamivir. With DNY ligand has the lowest ΔG_{binding} value at -7.8544 kcal/mol and pKi of 5.7243. Moreover, DNY has the highest number of interacted amino acid residues (thirteen interactions) and hydrogen bonds formed (six bonds). Therefore, further studies are needed on ligand affinity changes in the enzyme catalytic side sites and ADMET processes of ligands to metabolism in the body and taking into account the possibility of mutations in enzymes due to ligand binding.

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