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In Silico Study of Single Chain Fragment Variable Antibody and Indonesian Serotype-2 NS1 Dengue Virus Antigen

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***Abstract*—Objectives: The aim of this study was to determine the interaction between Indonesian NS1 DENV-2 with 6D4B10 and 8A6F2 single chain fragment variable antibodies. Method: In this study, bioinformatics approach was performed on the six structures of Indonesian NS1 antigenes of DENV-2 to obtain the best model using BLAST, Pyre2, Modeller 9.20 and the FireDock server for molecular docking. Results and Discussion: We obtained the binding energy of 6D4B10 antibody to NS1 D2/ID/SMG-SE001 was -55.04 kcal/mol and 8A6F2 antibody to D2/ID/JKT-J002 was -49.42 kcal/mol. The interactions of antigen-antibody complex were hydrogen bonds, electrostatic interactions, hydrophobic interactions and several other interactions. Conclusion: The results showed that 6D4B10 and 8A6F2 antibodies can be provide for designing molecular antibody fragments as one of dengue diagnostic kits agent in Indonesia.**

Keywords: dengue virus, antigen, antibody, molecular docking

I. INTRODUCTION

Dengue Haemorrhagic Fever (DHF) is one of infectious diseases caused by dengue virus that attacks many people in the world [12]. In 2011, World Health Organization reported that 50 million of the world's population was infected with DHF and 2.5% of them had been died. There were 59,047 cases of dengue fever in 2017 with the number of deaths was 444 people [5].

Clinical manifestation of DHF disease generally often resemble with the other diseases such as malaria, typhoid, and chikungunya. The fast and precise diagnosis of dengue virus infection becomes an important basic to handling dengue fever [12]. Laboratory test using IgG or IgM for diagnosis can only be done after five days after infection because the antibodies have just formed [3]. Early detection of viruses using non-structural protein 1 (NS1) more quickly detect dengue virus infection because they appeared on the first day of infection [4]. Rapid detection of dengue virus infection can prevent severe conditions in order to accurately distinguished dengue infection from other acute febrile illnesses [4].

Dengue fever caused by dengue virus of the genus Flavivirus, consists of single-stranded RNA. There are four serotypes namely DENV-1, DENV-2, DENV-3 and DENV-4 [12]. The structure consists of core protein (C), membrane protein (M), envelope protein (E), and seven non-structural proteins which is involve in replication of virus RNA [8]. Dengue virus transmitted through the *Aedes aegypti* and *Aedes albopictus* mosquitoes in Indonesia [1]. NS1 is the most *conserved* glycoprotein among other non-structural glycoproteins and also has a specific epitope that can distinguish dengue virus from the other flaviviruses [4].

NS1 is the most immunogenic among the other non-structural proteins [2]. NS1 is a glycoprotein produced in the endoplasmic reticulum as a hydrophilic monomer, glycosylated in the golgi body into a hydrophobic homodimer to be carried to the plasma membrane and then secreted into the bloodstream [9]. Therefore antigen examination from NS1 can be used for specific early detection of dengue virus infection [4].

According to the research of Sasmono et al., (2018) the most widespread of dengue virus in Indonesia is DENV-2. The sensitivity of dengue virus NS1 detecion in Indonesia is still low at only 49.2% (Noor et al., 2015) and for DENV-2 is only 68.4% [1]. The results of Gelanew and Hunsperger's research (2018) states that the presence of monoclonal antibodies that can be used as NS1 antigen detection were 6D4B10 and 8A6F2. However, there are no interactions studies between 6D4B10 and 8A6F2 monoclonal antibodies with NS1 DENV-2 antigens in Indonesia. In silico approach such as molecular docking is one of the important step that can be used to identify monoclonal antibody targets in DENV [7]. Based on the background, the aim of this research was to study the interaction between 6D4B10 and 8A6F2 monoclonal antibodies with Indonesian NS1 DENV-2 antigens in order to provide new information to design more specific antibodies against dengue viruses in

Indonesia so that this virus can be detected more effectively and efficiently.

II. MATERIAL AND METHOD

A. *Materials*

The *in silico* experiments were carried out using Biovia Discovery Studio software, Rosetta server (http://rosettaserver.graylab.jhu.edu/), Ellipro server (http://tools.iedb.org/ellipro/), Procheck server (https://servicesn.mbi.ucla.edu/PROCHECK/), Phyre2 server (http://sbg.bio.ic.ac.uk/phyre2), PatchDock server (https://bioinfo3d.cs.tau.ac.il/PatchDock/), and FireDock server (http://bioinfo3d.cs.tau.ac.il/FireDock/php.php).

The amino acid sequences of six Indonesian NS1 DENV-2 [1] retrieved from the NCBI website (http://www.ncbi.nlm.nih.gov/), thus the amino acid sequences of two single chain variable fragments 6D4B10 and 8A6F2 antibodies from Gelanew & Hunsperger (2018).

B. *Methods*

The amino acid sequences were performed BLAST (Basic Local Alignment Search Tools) at the GenBank site pages of NCBI by clicking "Run BLAST" with the "blastx" option. The *template* searched was done by accessing the Phyre2 website (http://www.sbg.bio.ic.ac.uk/phyre2) by entering amino acid sequences obtained from NCBI. The best template was selected from the BLAST and Phyre2 results based on the sequence similarity, high homology level, folding pattern, and stucture quality parameters.

Modeling constructed were evaluated by Ramachandran plot using Prochek server (https://www.ebi.ac.uk/thornton-srv/databases/pdbsum/Generate.html). A statistical potential energy to assess model, Discrete Optimized Protein Energy (DOPE) value, were calculated for the structures of model and template.

Antibodies modeling were done by accessing the server http://rosettaserver.graylab.jhu.edu/ with the antibody protocol. Antigen epitope prediction were done by accessing the ElliPro server (http://tools.iedb.org/ellipro/) to get B-cell epitope prediction. The *docking* process was done by the PatchDock server.

III. RESULTS

A. *Determination of Indonesian DENV-2 NS1 Template*

Amino acid sequences were obtained from the National Center for Biotechnology Information website (https://www.ncbi.nlm.nih.gov) by entering the amino acid sequences code of NS1 DENV-2 GenBank from the research of Aryati et al. (2013).

The results for the BLAST program are Chain A Dengue Type2 Non-structural protein 1 (PDB ID 4O6B_A), Chain A West Nile Virus Non-structural protein 1 (PDB ID 4O6C_A), Chain B West Nile Virus Non-structural protein 1 (PDB ID 4O6D_B), and Chain A Zika virus non-structural protein 1 (5K6K_A). All of the protein structures have a 100% query cover, that states the template coverage of the amino acid sequence of the Indonesian NS1 DENV-2 antigen. The results of the comparison templates can be seen in Table 1.

The Phyre2 result showed that the 5K6K was the best template for Indonesian NS1 DENV-2 amino acid sequences. The 5K6K_A template was able to cover the Indonesian NS1 DENV-2 amino acid sequence also, while in the 4O6B_A template, there is a missing amino acid sequence section. In addition, the 5K6K_A template has a higher resolution than other templates. Therefore, the 5K6K_A was chosen as the template for modeling the Indonesian NS1 DENV-2.

TABLE 1: COMPARISON OF INDONESIAN NS1 DENV-2 *TEMPLATES* ON BLAST AND PHYRE2

Parameter	PDB ID			
	4O6B_A	**5K6K_A**	**4O6C_B**	**4D6D_B**
Max score	718	429	450	429
Identity	96.88%	54.83%	55.97%	55.97%
E-value	0.0	2e-150	1e-158	3e-158
Confidence	100%	100%	100%	100%
Organism	Dengue virus type 2 (Thai strain / 16681/1984)	Zika virus	West Nile virus (strain NY-99)	West Nile virus (strain NY-99)
Family	Flaviridae	Flaviridae	Flaviridae	Flaviridae
Classification	Viral Protein	Viral Protein	Viral Protein	Viral Protein
Deposited	2013-12-20	2016-05-24	2013-12-20	2013-12-20
Released	2014-02-19	2016-07-06	2014-02-19	2014-02-19
Origin	Thailand	-	-	-
Resolution (Å)	3.0005 Å	1.89 Å	2.7508 Å	2.5936 Å
R-value (free)	0.217	0.189	0.232	0.191
R-value (work)	0.185	0.149	0.215	0.163

B. *Modeling of Indonesian DENV-2 NS1*

Modeling of six structures of the Indonesian NS1 DENV-2 antigen has been done using the Modeller 9.20 program with 5K6K as a template. The six antigen structures were named for each region D2/ID/JKT-J002, D2/ID/JKT-J004, D2/ID/MDN-M004, D2/ID/MDN-M022, D2/ID/SMG-SE001, and D2/ID/SUB-0011. Each structure was made up of five models and then selected the lowest Discrete Optimized Protein Energy value to be evaluated. The DOPE value indicates the potential energy of the model, the smaller DOPE value, the better quality of the model.

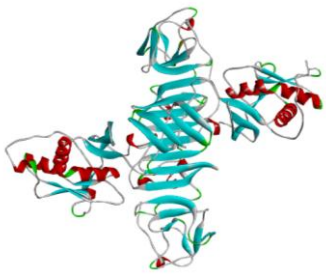


Fig. 1. Modeling Result of D2/ID/JKT-J-002

The results of the Ramachandran plot model of D2/ID/JKT-J002, D2/ID/MDN-M022, and D2/ID/SUB-0011 there was one amino acid in the forbidden region so it must be optimized in order to get a better structural model. Optimization was carried out to the amino acids in the forbidden region to stabilized the conformation of amino acid. After optimization, the model was re-evaluated through the Ramachandran plot. The optimization results showed that the quality of the model was better because there were no amino acids in the forbidden part and the percentage value in the permitted region more than 90%.

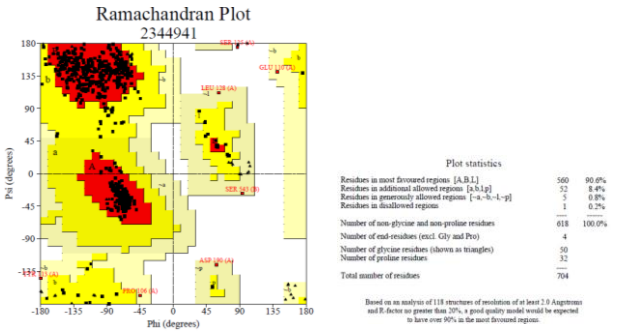


Fig. 2. The Ramachandran Plot Value of D2/ID/JKT-J002

Model evaluation was also carried out by comparing DOPE profile of models and template. DOPE profile of the model and template have a good quality, whereas the energy of each amino acid model was similar to the energy of the template. (Fig. 3.). Therefore, the results of modeling the Indonesian NS1 DENV-2 structures were continued to the molecular docking stage.

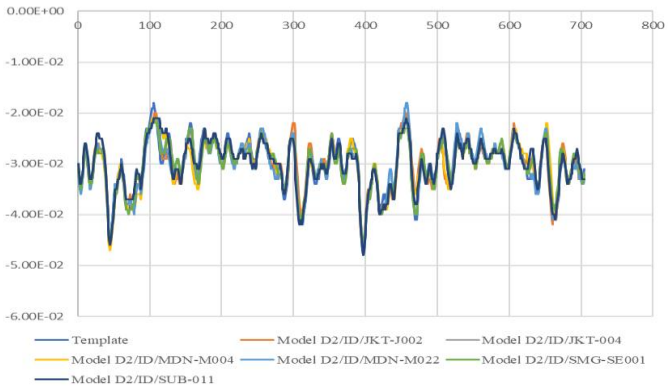
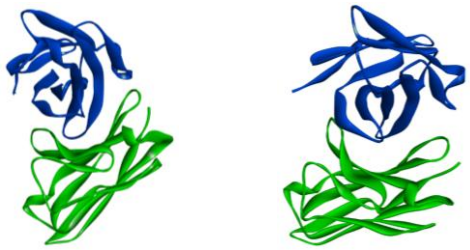


Fig. 3. Comparison of DOPE Profiles from Indonesian NS1 DENV-2 Models with the 5K6K Template

C. Modeling of 6D4B10 and 8A6F2 antibodies

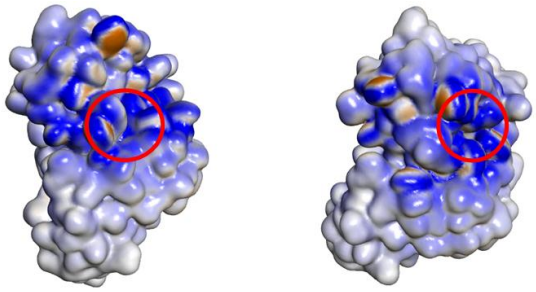
The amino acid sequence of 6D4B10 and 8A6F2 antibodies was obtained from the research of Gelanew and Hunsperger (2018). The antibodies being modeled were single-chain Fragment variable antibodies. Antibody modeling was performed using the Rosetta server. Rosetta has a special choice of antibody to produce a more specific quality model.



(a) (b)

Fig. 4. Modeling results of (a) 6D4B10 and (b) 8A6F2 antibodies; light chains (green) and heavy chains (blue)

The result of the 6D4B10 Ramachandran plot showed that threonine was in the not allowed region and the Ramachandran plot of 8A6F2 showed there were two amino acids (Threonine and Tyrosine) in restricted areas.



(a) (b)

Fig. 5. Surface Aromatic Clusters of (a) 6D4B10 and (b) 8A6F2 Antibodies

D. Epitope Analysis

The results of epitope mapping from Indonesian NS1 DENV-2 antigens have 11 parts of linear epitopes and 6 parts of discontinuos epitopes. The conserved epitope was found in the linear epitope, shown in Table 2.

TABLE 2: THE RESULTS OF INDONESIAN NS1 DENV-2 EPITOPE ANALYSIS

Amino Acid	Amino Acid Sequence	Residue	Score
278-322	DFCEGTTVVVTEDCGNRGPSLRTTT ASGKLITEWCCRSCTLPPLR	45	0789
69-92	KQITPELNHILSENEVKLTIMTGD	24	0.749
160-164	FGVFT	5	0.647
389-409	ESPSKLASAIQKAHEEGICGI	21	0.672
512-516	FGVFT	5	0.629

E. Molecular Docking of Antigen-Antibody

Docking using PatchDock server produced ten of the best antigen-antibody *docking* complexes. PatchDock is a rigid-body docking server that can only tether a molecule without regard to the flexibility of the molecule itself, so the results of the antibody-antigen complex obtained were evaluated using the FireDock server.

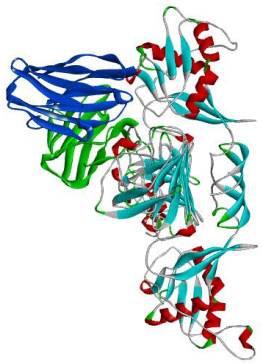


Fig. 6. Antigen-Antibody Complex of D2/ID/JKT-J002 with 6D4B10

The scoring results using FireDock server for each antigen and antibody are shown in Table 3. The lowest of Indonesian NS1 DENV-2 antigen-antibody binding energy were -55.04 for 6D4B10 with D2/ID/SMG-SE001 and -49.24 for 8A6F2 with D2/ID/JKT-J002.

TABLE 3: EVALUATION RESULTS OF FIREDOCK SERVER VALUES

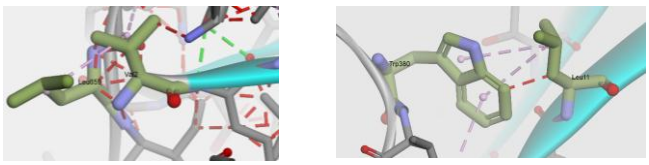
Antibody	Antigen	*Docking* Results	*Global Energy* (kcal/mol)	*Attractive VdW* (kcal/mol)	*Repulsive VdW* (kcal/mol)	ACE	*H-bond energy* (kcal/mol)
6D4B10	D2/ID/JKT-J002	Model 7	-35,40	-31.08	7.93	13.91	-3,37
	D2/ID/JKT-J004	Model 1	-31,54	-27,24	16.44	11.00	-1.36
	D2/ID/MDN-M004	Model 2	-51,57	-37.49	8.13	10.96	-5.05
	D2 / ID / MDN-M022	Model 3	-9.52	-25.82	51.57	14.51	-4.50
	D2 / ID / SMG-SE001	Model 5	-55.04	-35.49	16.85	13.07	-1.82
	D2 / ID / SUB-0011	Model 4	-22.91	-9.92	4.40	-5.10	-0.84
8A6F2	D2 / ID / JKT-J002	Model 7	-49.42	-37,65	26.04	.87	-5.25
	D2 / ID / JKT-J004	Model 5	-34.94	-19.79	6.18	12.62	-3.97
	D2 / ID / MDN-M004	Model 7	-29.46	-26.21	13.53	12.49	-5.82
	D2 / ID / MDN-M022	Model 5	-15.49	-14.32	4.51	8.93	-3.05
	D2 / ID / SMG-SE001	Model 3	-22,15	-27.42	59.34	14.50	-4.57
	D2 / ID / SUB-0011	Model 10	-23.08	-20.81	9.23	-0.13	-2.42

F. Docking Analysis

Interactions of each antigen-antibody complex were evaluated including hydrogen bonds, electrostatic interactions, hydrophobic interactions, and other interactions through the application of Biovia Discovery Studio. An evaluation of the number of antigen-antibody interactions shown in Table 4.

TABLE 4: EVALUATION ANTIGEN-ANTIBODY INTERACTION

Antibody	Antigen	Number of			
		Hydrogen Bonds	Electrostatic Interactions	Hydrophobic Interactions	Other Interactions
6D4B10	D2 / ID / JKT-J002	7	5	4	0
	D2 / ID / JKT-J004	4	4	5	0
	D2 / ID / MDN-M004	4	3	5	0
	D2 / ID / MDN-M022	13	5	10	0
	D2 / ID / SMG-SE001	7	1	6	0
	D2 / ID / SUB-0011	3	1	2	0
8A6F2	D2 / ID / JKT-J002	12	3	4	2
	D2 / ID / JKT-J004	8	4	0	0
	D2 / ID / MDN-M004	8	2	2	0
	D2 / ID / MDN-M022	6	2	0	0
	D2 / ID / SMG-SE001	5	7	6	1
	D2 / ID / SUB-0011	3	0	3	0



(a) (b)

Fig. 7. Unfavorable bond (red Line) of (a) D2/ID/MDN-M022 and (b) D2/ID/SMG-SE001

IV. DISCUSSION

The Ramachandran plot was used for structural validation by comparing the structural conformation with the secondary protein structure coordinates that have been modeled. The Ramachandran plot is divided into two major region were most favored region and the disallowed region. Amino acids in the most favored region sterically allow the values of ϕ (phi) and ψ (psi). Whereas the disallowed region was the part other than most favored which should only be occupied by glycine and proline plots. Certain combinations of ϕ (phi) and ψ (psi) are allowed because they will cause overlap between the atoms in the protein. Glycine plots may in the disallowed region because these amino acids do not have side chains,

whereas proline has cyclic side chains formed with carbonyl groups other than carboxyl groups in the main chain [11].

The binding site of the antibody is edited based on the aromatic group. Aromatic groups are charged residues so that they are more often observed as paratopes. Display of search results with aromatic groups are marked with a blue part, the fading of the color the number of aromatic groups decreases. These results will make it easier to observe the results of *molecular docking* to determine the best pose for binding antigen-antibodies.

Epitope prediction is an activity of determining the antibody-antigen binding site of a particular antigen. Estimates of parts that are epitopes are based on hydrophilicity, accessibility, flexibility and secondary structures. The Indonesian NS1 DENV-2 conserved epitope in the models will become the binding site of the antibody. This epitope mapping used to find out the antigen binding site with antibody so that it can be used as a basic for making vaccines or certain diagnostic kits. Scoring value from FireDock server is the based on calculation of Van der Waals interactions, Atom Contact Energy (ACE), electrostatic interactions, and predictions of additional bonds.

Hydrogen bonds that occur in antigen-antibody complex are divided into three types, salt bridges, conventional and carbon-hydrogen. Hydrogen bonds are interactions between particles that are strong enough to affect the value of bond energy.

The highest number of hydrogen bonds was found in complex of 6D4B10 and D2/ID/MDN-M022, but on the FireDock server the complex has the lowest bond energy compared to all models. After the interaction analysis, there were 279 unfavorable bonds in 6D4B10 and D2/ID/MDN-M022 complex. The number of unfavorable bonds can affect bond energy because it prevents the nucleophile from approaching the substrate. Whereas in the 6D4B10 and D2/ID/SMG-SE001 complex only 4 unfavorable bonds were found so that they did not affect the interaction of the atoms.

Based on the binding energy value and interaction analysis from the antigen-antibody complex, the best binding were 6D4B10 with D2/ID/SMG-SE001 and 8A6F2 with D2/ID/JKT-J002. Interactions involved in determining the bond energy value of hydrogen bonds, hydrophobic interactions, electrostatic interactions and other interactions.

V. CONCLUSION

Based on the research, it can be concluded that the 6D4B10 antibodies bind to Indonesian NS1 D2/ID/SMG-SE001 antigen with binding energy was -55.04 kcal/mol and 8A6F2 antibodies bind to D2/ID/JKT-J002 with binding energy was -49.42 kcal/mol. This research can provide new information about antibody candidates for the Indonesian NS1 DENV-2 detection kit component.

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