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Polytope Prediction for Dengue Vaccine Candidate Based on Conserved Envelope Glycoprotein of Four Serotypes of Dengue Virus and Its Antigenicity

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ABSTRACT

Dengue fever reported endemic in tropical and sub-tropical country. Dengue fever caused by dengue virus, has Envelope protein that often used for vaccine development to prevent the virus infection. Vaccine development to prevent four serotype dengue virus infection still unavailable. This study aims to design polytope from four conserved epitopes of dengue virus envelope glycoprotein to prevent infection of heterotypic dengue virus and predict its antigenic challenge by molecular docking. We investigate molecular modeling of polytope, immunoinformatics analysis of polytope, protein structure of antibodies, molecular docking and protein-protein docking assessment. The polytope categorized as a stabil protein with index 29.72, has molecular weight 6,139 kDa, has three exposed antigenic determinants region and has estimated half-life is: 3.5 hours (mammalian reticulocytes, in vitro),10 min (yeast, in vivo), and >10 hours (Escherichia coli, in vivo). The Polytope binds with four broadly neutralizing antibodies of B7, C8, A11, and C10 (bnAbs) which estimated that four bnAbs can recognize four serotypes of dengue virus. The designed polytope has prospect to produce in Escherichia coli and can be applied as vaccine of heterotypic dengue virus serotype. Polytope is potentially able to generate humoral and cellular immunity.

Key word: bnAnbs, polytope, dengue virus, binding energy, residues

INTRODUCTION

Dengue virus infection annually occurs in tropical country moreover it happened in sub-tropical country [1], [2], [3], [4]. Dengue virus (DENV) is consisted of four serotypes, members of family flaviviridae, genus Flavivirus [5], [6]. The incidence of dengue virus infection still be serious in the worldwide issues. The disease becomes endemic in more than one thousand countries [5]. The primary infection is most often asymptomatic, but secondary infection with different serotype can enhanced involving dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). The time interval between heterotypic DENV infections is another parameter influencing the magnitude of antibody-dependent enhancement (ADE) [5].

Despite intensive research, there is currently vaccine to prevent dengue infection but it unavailable to cover four serotypes [7], [8], [9], [10], [11]. The structural protein of the virus

such as envelope (E) glycoprotein of the virus membranes is the most exposed structure as principal target of neutralizing antibodies and has affects host cell receptor binding [12], [13], [14]. The glycosylation of E protein pattern differs according to DENV serotype. The degree and position of N-linked glycans affect the antigenic properties of DENV. The antigenic determinants of a number of biologically important substances consist of carbohydrates [15], [13]. E protein is the target for antigenic region to determined vaccine development today. Multi-epitope vaccine, known as polytope vaccine, is one of the best way to vaccine design [16], [17]. A potential vaccine must provide a delicate balance between the level of immunogenicity and generating response of class-I and –II Major Histocompatibility Complex (MHC) [5]. Based on Fitriyah [18], we selecting and combining conserved epitope that was analyzed from 629 sequences of DENV (E) glycoprotein of National Centre of Biotechnology Information database. The potential vaccine could be predicted its antigenicity when interacted with antibody in host's cellular system through in silico approach. Some specific antibody has found such as four broadly neutralizing antibodies (bnAbs) that reported can recognize four serotypes of dengue virus [29]. Thus, bnAbs can be directed docked with designed polytope. This study focus on designing protein polytope from conserved DENV E glycoprotein serotype 1-4, analyzing polytope antigenicity, and comparing interaction and binding energy between antibodies to recognize the polytope by bioinformatics.

EXPERIMENT

Molecular Modeling of Polytope

Four selected epitopes from Fitriyah [18] were used to design into a polytope. Each epitope joined by G S spacer. Each was separated from its neighboring epitope by a glycine (G) and a serine (S) codon to minimize interference between adjacent epitopes [17]. I-TASSER online software was used to model and evaluate the secondary and tertiary structure of polytope [19], [20], then visualized by Accelrys Discovery Studio 4.0.

Immunoinformatics Analysis of Polytope

The B cell epitopes of polytope were predicted using program Predicting Antigenic Peptides (http://imed.med.ucm.es/Tools/antigenic.pl), program IEDB Analysis Resources (http://tools.immuneepitope.org/tools/bcell/iedb) then chosen method with Kolaskar & Tongaonkar Antigenicity [21], [22]. Other features such as flexibility, hydrophilicity predicted using program BCEPred (http://www.imtech.res.in/raghava/bcepred/) and the polytope Estimating half-life based on ProtParam tool (http://web.expasy.org/protparam/) [23].

Protein Structure of Antibodies

Four antibodies (bnAbs) in complex with DENV 2 antigen downloaded from PDB database (http://www.rcsb.org/pdb/home/home.do) with accession number: 4UT6, 4UTA, 4UTB, and 4UT9. The binding site of antigen in the antibody was determined by using KFC (Knowledge-based FADE and Contacts) web server [24], [25]. The protein structures of antibodies were visualized with Vega ZZ software then the antigen was cleared from the antibody. The B7, C8, A11, and C10 is a broadly neutralizing antibody (bnAbs) that able to bind with four serotypes of dengue virus.

Molecular Docking

Direct docking between polytope and antibody were examined by PATCHDOCK (http://bioinfo3d.cs.tau.ac.il/PatchDock/index.html). The result displayed with FireDock then selected the best result based on the global energy value [26], [27]. Protein complex from docking result was visualized by Accelrys Discovery Studio 4.0.

Protein-protein Docking Assessment

Ligplot software was used to determine the interaction between antibodies and polytope then selected dimplot to analyze the interaction. Ligplot software was used to examine amino acid residues that involve in binding to hydrogen and hydrophobicity bond in the complex. The residues in binding site are mapped in 3D structure. We also compare the similar binding position and amino acid residue from each complex polytope-antibody with native antigen-antibody.

RESULT AND DISCUSSION

Polytope construction

The polytope amino acid was designed to encode four B cell epitopes E glycoprotein of dengue virus that generated response of MHC class-II. Each single epitope in tandem were connected with G (glycin) S (serin) spacer. Total length of polytope is 66 amino acids. The secondary structure of epitopes propensity consists of a coil, beta sheet, and alpha helix. Three-dimensional protein models of polytope consist of the beta sheet-coil-beta sheet-coil-beta sheet-coil-beta sheet that presented in exposed surface (Figure 1).

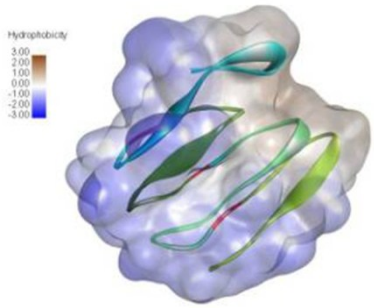

Figure 1. (Right) The tertiary structure of polytope that consist of four serotypes of dengue virus that connected by G S spacer (red colour).

Immunoinformatics Analysis of Polytope

The polytope has flexibility and hydrophilicity. The epitope region that has flexibility and hydrophilicity shows on Table 1. Antigenicity of the polytope shows three antigenic determinant region based on IEDB (Figure 2) and program Predicting Antigenic Peptides (Table 2). It known that some different position of the sequences that IEDB result predict GSALTGA, SSALAGATEV, HTALTGA as antigenic determinat region (threshold:0,977) but program Predicting Antigenic Peptides result predict that GGSALTG, GSSALAGATE, SHTALTG as antigenic determinant region (threshold:0,978). We decide that the antigenic region position are GSALTG, SSALAGATE, HTALTG. Antigenicity of polytope indicates that polytope could be interacted or recognized with antibody in host's cellular system. Protein structure of polytope has His (H) in N-terminal of the sequence. The instability index (II) is computed to be 29.72 that is classifies the protein of polytope as stable with size 6,139 kDa. The estimated half-life of polytope is 3.5 hours (mammalian reticulocytes, in vitro), 10 min (yeast, in vivo), and >10 hours (*Escherichia coli*, in vivo).

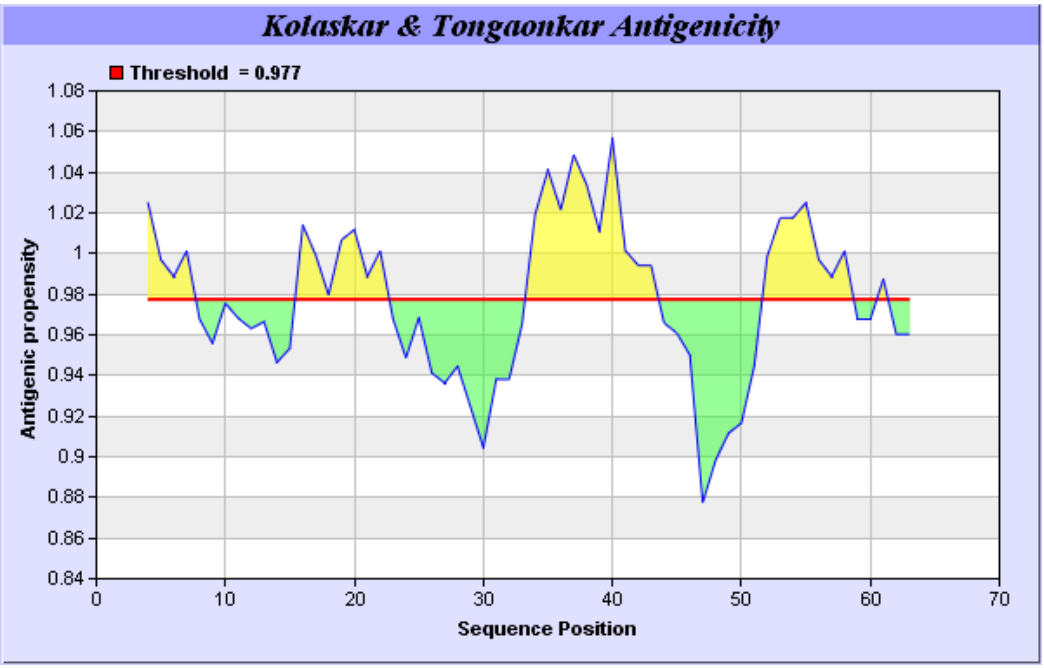

Figure 2. B cell epitope prediction with IEDB. Green regions under threshold color denotes unfavorable regions. Yellow colors are above the threshold sharing higher scores related to the properties of interest. Horizontal red line is the threshold.

Table 1. Prediction of Polytope based on different parameters by BcePred, IEDB[1], and program Predicting Antigenic Peptides[2] software.

Prediction Parameter	Sequence position
Flexibility	(8-16), (23-34), (41-51)
Hydrophilicity	(11-17), (26-36), (39-54)
Antigenicity	(16-22), (34-43), (52-58)[1]
	(15-21), (33-42), (51-57)[2]

Protein Docking between antibody with polytope

Docking result shows that polytope can bind four antibodies based on its binding energy, hydrogen bond (Table 2) and hydrophobic bond. Polytope binds chain H of B7 bnAbs, chain I and M of C8 bnAbs, chain H and L of A11 bnAbs, and binds chain I of C10 bnAbs (Figure 3). B7 bnAbs binds at nine amino acid residues of polytope with binding energy -27,51 kJ/mol. C8 bnAbs binds at 16 amino acid residues of polytope with binding energy -43,87 kJ/mol. In other hand, A11 bnAbs binds at 19 polytope's amino acid residues with binding energy -26,19 kJ/mol. The complex C10 bnAbs bind at 15 amino acid residues of polytope with binding energy -45,77 kJ/mol. We found that complex C10 antibody with polytope had the highest binding energy.

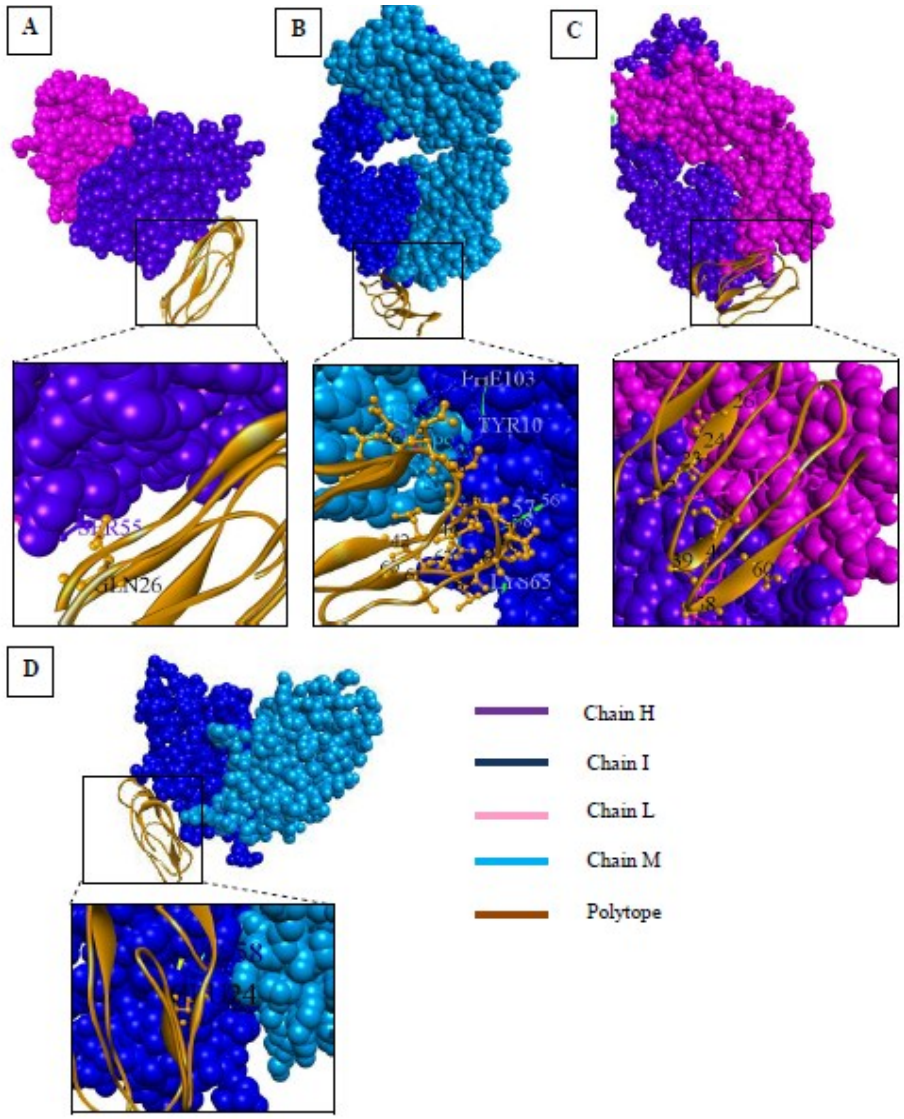

Figure 3. Molecular Docking interaction between Polytope and bnAbs. The complex of polytope interaction with B7 bnAbs (A), C8 bnAbs (B), A11 bnAbs (C), and C10 bnAbs (D).

Three-dimensional structure the complexes of bnAbs and polytope show the same orientation and binding position compared to the antibody-native antigen complex from the previous study based on Rouvinski [29] that presented in Figure 3. The highest total number of hydrogen bonds occurs in a complex of polytope with C8 bnAbs and the lowest in a complex of polytope with C10 bnAbs (Table 2). It determined that complex protein polytope with C8 bnAbs more stabil. It stabilized by relatively weak interactions by hydrogen bonds between main chain atoms. Its strength depends on distance and also on the bond angle [28]. There are some residue that has same position based on previous study by Rouvinski [29]. These one amino acid residue of complex with B7 bnAbs (^{55}Ser), eight amino acid residue of complex with C8 bnAbs (^{56}Asp, ^{57}Ser, ^{58}Ala , ^{65}Lys, ^{103}Phe, ^{104}Tyr, ^{93}Asn, ^{94}Trp), three amino acid residue of complex with A11 bnAbs (^{55}Ser, ^{56}Thr, ^{95}Arg), and one amino acid residue of complex with C10 bnAbs (^{58}Lys) that presented in Figure 3. Total number hydrophobic bond of polytope with B7 bnAbs are 14 bonds, polytope with C8 are 26 bonds, polytope with A11 bnAbs are 26 bonds, and polytope with C10 bnAbs are 25 bonds.

Table 2. Hydrogen bond interaction between polytope and four antibodies

interaction	Point interaction	Antibody's chain	Distance (amstrong)	Global energy
Polytope-B7 bnAbs	Glu 42-asp 72	H	2,74	-27,51
	Gln 26-Asp 53	H	2,74	
Polytope-C8 bnAbs	Ser 45-Tyr 104	I	2,67	-43,87
	Gln 62-Tyr 60	I	2,52	
	Gln 62-Tyr 60	I	2,32	
	Glu 24-Asn 93	I	2,35	
Polytope-A11 bnAbs	Thr 23-Arg 95	L	2,36	-26,19
	Glu 24-Thr 93	L	2,76	
	Asp 44-Ser 94	L	2,70	
Polytope-C10 bnAbs	Glu 42-Thr 57	I	2,11	-45,77

CONCLUSION

Polytope has three antigenic determinant region. Antigenic challenge of polytope were predicted through molecular docking with four bnAbs. The polytope can bind four antibodies that reported can recognize four DENV serotype. Molecular weight of polytope protein is 6,139 kDa and has longest half-life for more than 10 hour in *Escherichia coli*. Polytope has potential for further examination as a vaccine candidate and over expressed in *Escherichia coli.* Thus, protein production could be immunized to prevent infection of heterotypic from dengue virus serotypes.

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REFERENCES

[1] F. F., Amâncio, M. L., Ferraz, M. C. M., Almeida, Pessanha, J. E. M., Iani, F. C. M., Fraga, G. L., Lambertucci, J. R., Carneiro, M., *J. Infect. Public Health* **2014**, *7* (6), 547–552.
[2] F. P., Rocha, H. S., Rodrigues, M. T. T. Monteiro, D. F. M., Torres, *Oper. Res. Health Care,* **2015**, *7*, 122–131.
[3] Lardo, S., Utami, Y., Yohan, B., Tarigan, S. M. M. U., Santoso, W. D., Nainggolan, L., Sasmono, R. T., *Asian Pac. J. Trop. Med.* **2016**, *9* (2), 134–140.
[4] Myat, M., Tun, N., Kyaw, A., Makki, N., Muthugala, R., Nabeshima, T., Zin, K., Morita, K., *Infect. Genet. Evol.* **2016**, *43*, 31–37.
[5] Murrell, S., Wu, S., Butler, M. *Biotechnol. Adv.* **2011**, *29* (2), 239–247.
[6] Bäck, A. T., Lundkvist, Å., *Infect. Ecol. Epidemiol.* **2013**, *1*, 1–22.
[7] Johnson, J., Hunt, A. N. N. R., Bolin, A., Roehrig, J. T., Chu, M. A. Y. C. *Virology* **1990**, *675*, 668–675.
[8] Mart, J., Hermida, L., Alvarez, M., Vald, I., Chinea, G., Rosario, D., Guill, G., Guzm, G. *Virus Res.* **2006**, *121*, 65–73.
[9] Kelly, E. P., Puri, B., Sun, W., Falgout, B. *Vaccine* **2010**, *28* (17), 3030–3037.
[10] Rantam, F. A., Purwati, Soegijanto, S., Susilowati, H., Sudiana, K., Hendrianto, E., Soetjipto, *Trials Vaccinol,.* **2015**, 4, 75–79.
[11] Hunsawong, T., Sunintaboon, P., Warit, S., Thaisomboonsuk, B., Jarman, R. G., Yoon, I., Ubol, S., Fernandez, S. *Vaccine* **2015**, *33* (14), 1702–1710.

[12] Sánchez-burgos, G., Ramos-casta, J., Cedillo-rivera, R., Dumonteil, E. *Virus Res.* **2010**, *153*, 113–120.
[13] Cedillo-Barrón, L., García-Cordero, J., Bustos-Arriaga, J., Leon-Juarez, M., Gutierrez-Castaneda, B. *Microbes Infect.* **2014**, *16*, 711–720.
[14] Venkatachalam, R., Subramaniyan, V. *Asian Pacific J. Trop. Dis.* **2015**, *5* (Suppl 1), S47–S50.
[15] Paul, William E., M. *Fundamental Immunology. Fifth Edition*, Lippincott Williams & Wilkins: Maryland, 2003.
[16] Suhrbier, A. *Expert Rev. Vaccines* **2002**, 207–213.
[17] Han, K., Zhao, D., Liu, Y., Huang, X., Yang, J., Liu, Q., An, F., Li, Y. *Res. Vet. Sci.* **2016**, *104*, 174–180.
[18] Fitriyah. Insilico Analisys of Multi-Epitope Vaccine Candidat Design Againts Mannose Receptor to Inhibit Dengue Infection, University of Brawijaya, Malang, 2012.
[19] Zhang, Y., *BMC Bioinf.*, **2008**, *8*, 1–8.
[20] Yang, J., Yan, R., Roy, A., Xu, D., Poisson, J., Zhang, Y. *Nat. Publ. Gr.* **2015**, *12* (1), 7–8.
[21] Kolaskar, A., Tongaonkar, P. *FEBS Lett* **1990**, *276* (1-2), 172–174.
[22] Kim, Y., Ponomarenko, J., Zhu, Z., Tamang, D., Wang, P., Greenbaum, J., Lundegaard, C., Sette, A., Lund, O., Bourne, Nielsen, M., Peters, B. *Nucleic Acids Res* **2012**, *40*.
[23] Saha, S., Raghava, G. P. . *Proteins Struct, Funct Bioinf.* **2006**, *65*.
[24] Darnell, S. J., Page, D., Mitchell, J. C. *Proteins* **2007**, *68* (4), 813–823.
[25] Zhu, X., Mitchell, J. C. *Proteins* **2011**, *79* (9), 2671–2683.
[26] Duhovny, D., Nussinov, R., Wolfson, H., *Algorithms in Bioinformatics* **2002**, Proceedings of the 2nd International Workshop, WABI 2002 Rome, Italy, September 17-21, 2002185–200.
[27] Schneidman-Duhovny, D., Inbar, Y., Nussinov, R., Wolfson, H. J. *Nucleic Acids Res.* **2005**, *33*, 363–367.
[28] Lesk, A. M. *Introduction to Bioinformatics*, Oxford University Press: UK, 2002.
[29] Rouvinski, A., Guardado-Calvo, P., Barba-Spaeth, G., Duquerroy, S., Vaney, M., Kikuti, C. M., Sanchez, M. E. N., Dejnirattisai, W., Wongwiwat, W., Haouz, A., Girard-Blanc, C., Petres, S., E.Shepard, W., Despres, P., Arenzana-Seisdedos, F., Dussart, P., Mongkolsapaya, J., R.Screato, G., Rey, F. A. *Nature* **2015**, 1–16.