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Original Article

Designing a polytope for use in a broad-spectrum dengue virus vaccine

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الملخص

أهداف البحث: البروتينات السطحية لفيروس حمى الضنك غالبا ما تستخدم لتطوير اللقاح لمنع العدوى الفيروسية. ومع ذلك، الاختلاف العالي للبروتينات السطحية لأربعة أنماط مصلية لفيروس حمى الضنك تعقّد تطوير اللقاح الذي يمكن أن تغطي جميع سلالات الفيروس. تركز هذه الدراسة على تصميم بوليتوب لحاتمة محفوظة من أربعة أنماط مصلية لفيروس حمى الضنك ضد أجسام مضادة محايدة على نطاق واسع.

طرق البحث: قمنا بتركيب بوليتوب من أربعة حواتم محفوظة جنبا إلى جنب ترتبط مع إنزيم هيستيديل-تي آر إن أي المخلق كفاصل بين اثنين من الحواتم. استند هذا على بنية بروتين البوليتوب والموقع الجزيئي بين البوليتوب مع أربعة أجسام مضادة محايدة على نطاق واسع.

النتائج: ارتبط البوليتوب بدقة لأربعة أجسام مضادة محايدة على نطاق واسع. علاوة على ذلك، زاد البوليتوب تقارب موقع ربط الأجسام المضادة المحايدة على نطاق واسع بالمقارنة إلى مستضداد DENV2. وأظهر المركب بين بوليتوب والجسم المضاد أ١١ أدنى طاقة رابطة عند مقارنته للثلاثة الأجسام المضادة الأخرى. ووجد أعلى عدد من الروابط الهيدروجينية في البروتين المركب بين بوليتوب والجسم المضاد ب ٧. كما أظهرت الروابط الهيدروجينية لجميع المركبات البروتينية مسافات بين ٢.٠٧-٣.٠٣ أنغستروم، مما يعني أن الروابط الهيدروجينية وازنت البروتينات المركبة.

الاستنتاجات: تفاعلت البوليتوب المطورة مع أربعة أجسام مضادة محايدة على نطاق واسع وتعرفت على الأربعة الأنماط المصلية لفيروس حمى الضنك. توصي نتائج هذه الدراسة أن البوليتوب يحفز المزيد من التطور لإيجاد لقاح واسع السلالة لفيروس حمى الضنك.

الكلمات المفتاحية: حمى الضنك؛ أجسام مضادة محايدة على نطاق واسع؛ بوليتوب؛ نمط غير متجانس؛ بقايا

Abstract

Objectives: Dengue virus surface proteins are often used in the development of vaccines that protect against dengue virus infection. However, the surface proteins on the four serotypes of dengue virus display high variation, which increases the difficulty of developing a vaccine that can protect against all viral strains. In this study, a polytope that is recognized by broadly neutralizing antibodies (bnAbs) was designed using conserved epitopes from the four serotypes.

Methods: We constructed a polytope using four conserved dengue virus epitopes such that two aligned epitopes were separated from the other two epitopes by a histidyl-tRNA synthetase spacer. The epitopes were selected based on our previous docking studies. We then performed molecular docking of the polytope with the four bnAbs.

Results: The polytope bound precisely to the four bnAbs—B7, C8, A11, and C10. Moreover, the polytope had a higher affinity for the bnAbs compared to the DENV2 antigen. The polytope and A11 antibody complex had the lowest binding energy relative to complexes between the polytope and the other three antibodies assessed. The highest total number of hydrogen bonds was found in the polytope and B7 antibody complex. The hydrogen bond length in all the complexes ranged from 2.07 to 3.03 Å, implying that hydrogen bonds stabilized the complexes.

Conclusion: The developed polytope interacted with four different bnAbs that recognize the four serotypes of dengue virus. The results of this study suggest that this

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polytope warrants further development for use in a broad-spectrum vaccine against dengue virus.

Keywords: bnAbs; Dengue; Heterotype; Polytope; Residue

Introduction

The annual incidence of dengue fever continues to rise, particularly in the Asia–Pacific, Africa, and the Caribbean.[1–3] Dengue viruses belong to the *Flaviviridae* family. The mature virions contain non-structural proteins and the structural capsid (C), membrane (M), and envelope (E) proteins. There are four serotypes of dengue virus, where each is specifically recognized by host cells during infection. Infection with one serotype results in immunity against that serotype, but not against other serotypes.[4,5]

Secondary infection with a different dengue virus serotype can cause dengue haemorrhagic fever or dengue shock syndrome.[5] Therefore, significant effort has been made to prevent dengue infection, such as by decreasing virus virulence and developing vaccines protective against each dengue virus serotype.[6–8] In 2015, the World Health Organization (WHO) approved a new dengue vaccine, CYD-TDV, and several additional candidate vaccines are currently undergoing clinical development.[9] Several of these vaccines, including CYD-TDV, are live attenuated vaccines.[10–12] These live attenuated vaccines have associated risks because pathogens are used as the vaccinating agent. One recent study found that injection of polytopic live attenuated dengue virus enhanced B- and T-cell activation, but failed to lead to neutralizing antibody production.[13] Subunit viral proteins also have potential for use in vaccines. For example, a vaccine containing dengue virus E glycoprotein epitopes has been proposed.[14] However, potential vaccines are still rather limited because they would not be effective against all four serotypes of dengue virus.[6,7]

Using multiple conserved epitopes or polytopes is a common strategy used in vaccine design as it can stimulate immunity against many viral strains.[15,16] Therefore, we designed a polytope vaccine *in silico* from epitopes from all four dengue virus serotypes, which when presented with class I or II major histocompatibility complexes (MHC) could stimulate B and/or T cells. The designed polytope was based on conserved epitopes from each serotype extracted from 629 E protein sequences obtained from the National Centre of Biotechnology Information database.[17] This challenging polytope was designed using a bioinformatics approach to bind to broadly neutralizing antibodies (bnAbs).

Materials and Methods

This study was conducted from July 2015 to January 2016 at the Biocomputational Laboratory in the Biology Department, Brawijaya University, Malang, Indonesia.

Molecular modelling of the polytope

Four epitopes selected from our previous work[17] were used to design a polytope by aligning two epitopes and then joining them with another two epitopes using a linker derived from a region of histidyl-tRNA synthetase (GenBank: AEG33143.1), 319-GFGLPEEK-326. This linker peptide is highly conserved across species, forms part of the host cell response, and is hypothesized to fail to generate any immune or autoimmune responses.[16] I-TASSER online software was used to model and evaluate the tertiary structure of the polytope,[18,19] which was then visualized using Accelrys Discovery Studio 4.0.[20] The molecular weight of the polytope was estimated using the ProtParam tool (http://web.expasy.org/protparam).[21] The quality of the protein geometry was evaluated using ModFOLD version 3.0. The global model quality scores ranged from 0 to 1. The consistency of the global scores allowed calculation of p-values, which represent the probability that each model is incorrect.[22,23]

Antibody protein structures

The protein structures of the dengue virus-recognizing antibodies in complex with their cognate antigens with accession numbers 4UT6, 4UTA, 4UTB, and 4UT9 were retrieved from the Protein Data Bank. The antibody-antigen complexes were visualized using the Vega ZZ software, and the antigen was then removed from the antibody complexes. B7, C8, A11, and C10 are conserved bnAbs that recognize the four serotypes of dengue virus.[24]

Molecular docking

The binding affinities between the polytope and the four aforementioned bnAbs were examined using PATCHDOCK. The binding site of the polytope antigen on each antibody was determined using the Knowledge-based FADE and Contacts web server. The results were displayed using FireDock and then the best result was selected based on the global energy.[25,26] Protein complex docking results were visualized using Accelrys Discovery Studio 4.0.[20]

Protein–protein docking assessments

Ligplot software was used to assess the interactions between the four antibodies and the polytope. This software assessed the amino acid residues involved in the formation of hydrogen and hydrophobic bonds. The residues in the binding sites were then three-dimensionally mapped. Similar binding positions and amino acid residues in the polytope–antibody complexes were compared with native antigen–antibody complexes. The Ligplot program automatically generated 2D schematic representations of protein–protein and protein-ligand complexes. The output was a colour or black-and-white PostScript file containing a straightforward and informative representation of the intermolecular interactions, including hydrogen bonds and hydrophobic interactions, and their strengths and atom accessibility. This method has been used to analyse interactions in protein–protein and protein-ligand complexes.[27]

Results

Polytope construction

Four conserved dengue virus epitopes were predicted based on their secondary and tertiary structures. The secondary structures of the epitopes consisted of a coil and/or beta sheet. These epitopes represent the four dengue virus serotypes and were constructed in tandem using a linker as a spacer between two epitopes. Three-dimensional protein models of the polytope revealed a coil-beta sheet-beta sheet-coil on the exposed surface (Figure 1).

Quality of 3D model of polytope protein

Based on MoldFold predictions, the p-value and confidence of the polytope 3D model was $p < 0.05$, which is considered moderate confidence and implies that there is less than a 1/20 chance that this model is incorrect. The global model quality score was 0.2183, indicating that the polytope model was appropriate for assessing docking.

Pairwise docking of polytope and bnAbs

Using the same procedure, the B7, C8, A11, and C10 bnAbs were separately and directly docked with the polytope as their antigen. The antibody binding sites were in accordance with a previous study and determined using the Knowledge-based FADE and Contacts web server. In order to experimentally confirm recognition of the antigen by the antibodies, identify interchain hydrogen bonds, and calculate binding affinities between antibodies and antigen, we docked the polytope separately with the B7, C8, A11, and C10 bnAbs (Tables 1 and 2). Interfacial contact between the polytope residues occurred via interactions with either the heavy (H) or light (L) chains of B7, C8, A11, and C10 bnAbs (Table 1), as shown in Figure 2.

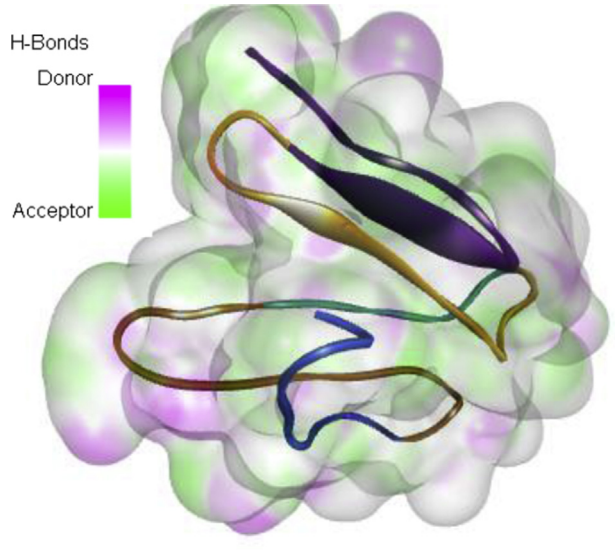

Figure 1: Tertiary structure of polytope consisting of epitopes from four dengue virus serotypes connected by a linker in the middle of the structure (green).

Docking of polytope with B7 bnAb

At the interface of the B7 bnAb-polytope complex, there are 41 hydrophobic (Table 1) and five hydrogen bonds (Table 2). The B7 bnAb-polytope complex contains the following hydrogen bonds: Ser 71-Glu 22, Tyr 110-Gln 36, Tyr 111-Glu 37, Ser 59-Gly 50, and Ser 99-Thr 58. The global binding energy of the B7 bnAb-polytope complex was −40.30. There were 13 interfacing residues on the polytope that interacted with the H chain and two that interacted with the L chain of the B7 bnAb. The polytope had B7 bnAb binding sites at Ser 56 and Lys 95 that are also the binding sites of the native antigen.

Docking of polytope with C8 bnAb

The C8 bnAb-polytope complex had 48 hydrophobic (Table 1) and two hydrogen bonds (Table 2). The interactions between the C8 bnAb and polytope included the following hydrogen bonds: Met 109-Lys 38 and Asn 102-Asp 48. The global binding energy of the complex was −46.03. There were 14 polytope interfacing residues when interacting with the H chain and three when interacting with the L chain of the C8 bnAb. The polytope bound to C8 bnAb at sites also bound by the native antigen, which were the C8 bnAb residues Tyr 100, Phe 103, Tyr 106, Asn 93, and Phe 32.

Docking of polytope with A11 bnAb

The interface between the A11 bnAb and polytope involved 11 hydrophobic (Table 1) and one hydrogen bonds (Table 2). The interactions between A11 bnAb and the polytope included one hydrogen bond, Asn 63-Ser 13. The global binding energy of the A11 bnAb-polytope complex was −47.07. There were four polytope interfacing residues when interacting with the H chain and three when interacting with the L chain of A11 bnAb. The polytope bound to A11 bnAb site that is also bound by native antigen, which was Tyr 100.

Docking of polytope with C10 bnAb

The C10 bnAb-polytope polytope contained 38 hydrophobic (Table 1) and three hydrogen bonds (Table 2). The interactions between C10 bnAb and polytope included hydrogen bonds: Thr 52-Gly 27, Ser 53-Thr 29, and Asn 31-Ser 49. The global binding energy of C10 bnAb with polytope was −38.30. There were 11 polytope residues interacting with the H chain and five interacting with the L chain of C10 bnAb. The polytope bound to C10 bnAbs at sites also bound by the native antigen, which were Tyr 100, Phe 30, Asn 31, Tyr 32, Asp 50, Thr 52, and Ser 53.

Discussion

Glycosylated envelope protein-mediated interactions occur between dengue virus and host cells. N-glycosylation of envelope proteins can promote proper folding and subsequent trafficking using host cell chaperones.[4,28] Host cell receptors involved in the immune response to dengue virus include laminin receptor, mannose receptors, such as the macrophage mannose receptor (MMR) and the dendritic

Table 1: Ligplot and PatchDock confirmation results.

Complexes		Interfacing polytope residues	Antibody chain (heavy chains: H & light chains: L)	Total number of hydrophobic bonds	Global energy (The binding energy of the molecules)
Polytope	B7 bnAb	Met 12, Ser 13, Ala 20, Glu 22, Gly 27, Ser 30, Phe 32, Gly 33, Leu 34, Gln 36, Glu 37, Gly 50, Asp 51	H	41	−40.30
		Asp 48, Asp 51	L		
	C8 bnAb	Met 12, Gly 19, Ala 20, Glu 22, Gly 31, Pro 35, Gln 36, Glu 37, Glu 46, Asp 48, Ser 49, Gly 50, Asp 51, His 54	H	48	−46.03
		Lys 38, Gly 67, Thr 68	L		
	A11 bnAb	Gly 27, Ser 49, Gly 50, Asp 51	H	11	−47.07
		Gln 11, Ser 13, Gln 24	L		
	C10 bnAb	Leu 4, Thr 5, Gly 6, Thr 55, Leu 57, Gly 59, Ala 60, Glu 62, Ile 63, Gln 64, Thr 65	H	38	−38.30
		His 1, Gly 27, Thr 29, Ser 49, Asn 53	L		

cell-specific ICAM-3 grabbing non-integrin (DC-SIGN), and CD14-associated protein.[28,29] This study focused on conserved dengue virus epitopes that were chosen based on our previously published work.[17] Epitopes were selected based on docking between the mannose receptor and E glycoproteins from all serotypes of the dengue virus. The epitopes were predicted based on their antigenicity when presented with class II MHC. Furthermore, the chosen epitopes were used to design a subunit vaccine. Subunit vaccines are potential substitutes for the current live attenuated dengue virus vaccine approved by the World Health Organization.[9] A recently published study found that this live attenuated vaccine stimulated B and T cells but did not increase production of neutralizing antibodies.[13] We combined these four epitopes into a polytope to create a candidate vaccine. This vaccine used B cell epitopes theorized to induce immunity against multiple antigenic targets and stimulate antibody production.[30–32] The polytope was composed of 68 amino acids, included a histidyl-tRNA synthetase linker, and had a molecular weight of 6.56 kDa.

Vaccines optimized to generate a high neutralizing antibody response reduce the frequency of symptomatic infections.[33] The dengue virus-specific polytope designed in this study consisted of epitopes that solely interact with bnAbs. The characterization of interactions between the polytope and binding antibodies in this work provides promising information regarding the potential of the polytope as a candidate bnAb-binding dengue virus vaccine candidate. To examine the antigenicity of this polytope, we analysed molecular docking of the polytope with four dengue virus-specific antibodies, C7, B8, A11, and C10. Further analysis found the polytope bound to the bnAbs with similar orientations and via several same residues as the native antigen, based on the binding sites proposed by Rouvinski et al. for complexes of native DENV2 and these bnAbs.[24] The matching antibody binding residues are presented in Figure 2. We found the polytope-A11 bnAb complex had the lowest binding energy of the complexes assessed. Meanwhile, the B7 bnAb-polytope and A11 bnAb-polytope complexes had the most and fewest hydrogen bonds, respectively (Table 2). Intermolecular hydrogen bonding is important for binding specificity in and stabilization of antibody-antigen complexes because it separates the individual proteins by minimum and maximum distances of 2 and 8.0 Å, respectively.[34] All the docking distances calculated in this study were between 2.07 and 3.03 Å (Table 2), where the hydrogen bonds functioned to stabilize the complexes.

Table 2: Hydrogen bonds between polytope and antibodies.

Interaction	Points of interaction	Distance (Angstrom)	Total number of hydrogen bonds
Polytope-B7 bnAb	Glu 22-Ser 71	2,85	5
	Gln 36-Tyr 110	2,07	
	Glu 37-Tyr 111	3,03	
	Gly 50-Ser 59	2,91	
	Thr 58-Ser 99	2,72	
Polytope-C8 bnAb	Lys 38-Met 109	2,84	2
	Asp 48-Asn 102	2,74	
Polytope-A11 bnAb	Ser 13-Asn 63	3,20	1
Polytope-C10 bnAb	Gly 27-Thr 52	2,90	3
	Thr 29-Ser 53	2,62	
	Ser 49-Asn 31	3,33	

The polytope was estimated to have an antigen-based role when interacting with the four antibodies via interfacing residues. The bnAb paratopes in the H and L chains were mostly exposed. Both the heavy and light chains of the B7 and A11 bnAbs had exposed paratopes that could make contacts with the polytope. In contrast, previously published reports have stated that the exposed paratopes of these bnAbs were located on the heavy chains, despite the light chain alone being in contact with N153 glycan when docked with native antigen.[24] Therefore, the three-dimensional structure of the polytope changes from a coil-beta sheet-beta sheet-coil structure into a coil-coil-coil-coil structure after docking with each of the antibodies assessed. This interaction illustrates the ability of the polytope to bind to bnAbs recognizing the four dengue virus serotypes and, thus, may provide protection against all four serotypes. However, this protection needs to be confirmed using *in vivo* assays and protein expression of the polytope in the selected host needs

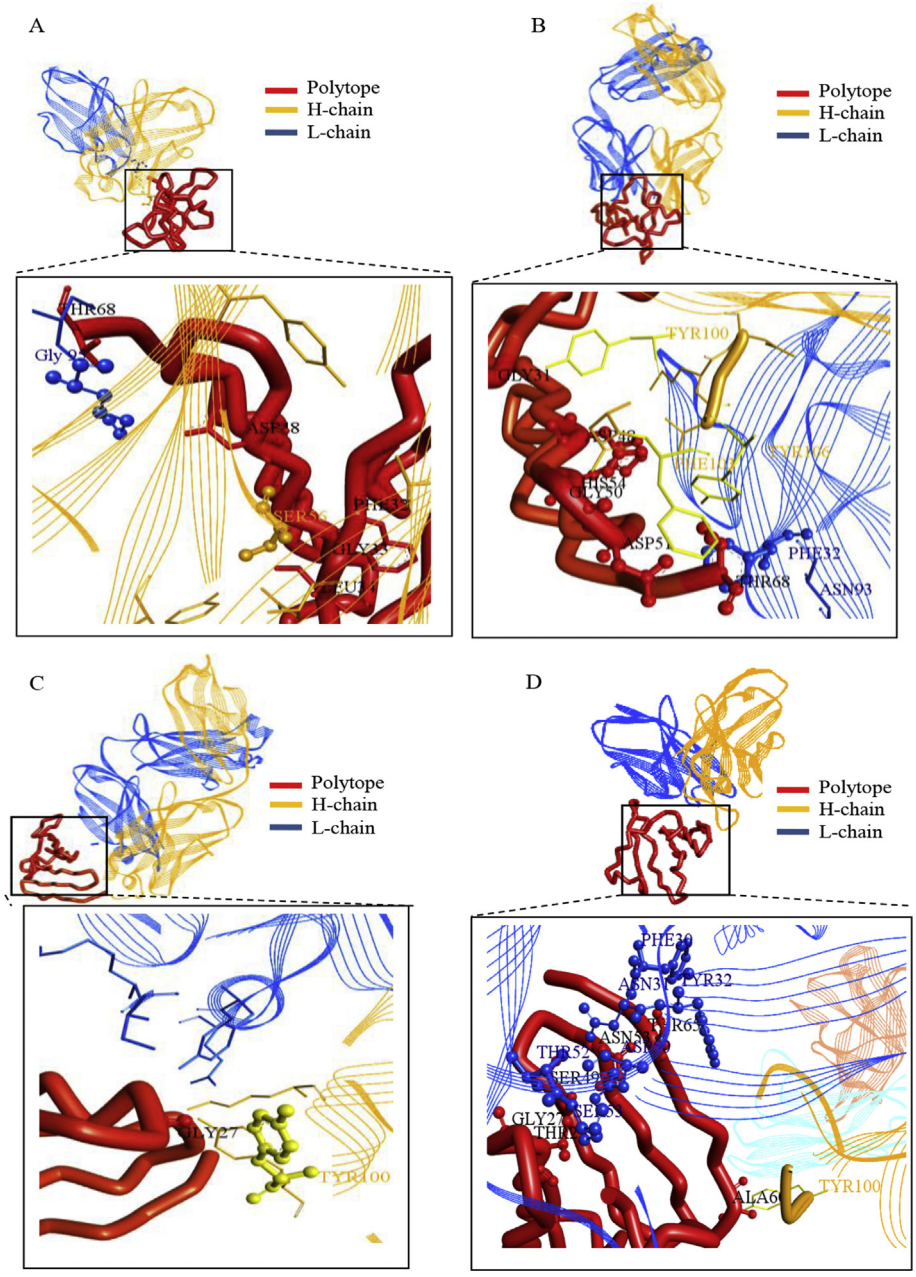

Figure 2: Molecular docking between polytope and bnAbs. Polytope interactions with (A) B7, (B) C8, (C) A11, and (D) C10 antibodies.

to be optimized. When designing recombinant proteins, the size of the polytope should be improved using protein tagging to minimize protein degradation in the host. Development of a polytope that interacts with antibodies involved in dengue virus attachment to mannose receptors is another potential area of further investigation.

Conclusion

Four conserved epitopes were successfully joined into a polytope using a linker derived from histidyl-tRNA synthetase. This polytope had good binding affinities for four antibodies that recognize four different dengue virus serotypes. Binding between the polytope and bnAbs indicated that the polytope may induce B-cell immunoglobulin production. Therefore, such polytope warrant further examination due to its potential for use in vaccine that protect against heterotypic infection with different serotypes of dengue virus.

Conflict of interest

The authors have no conflict of interest to declare.

Research involving human participants and animals

This article does not contain any studies on human participants or animals.

Ethics and informed consent

This study did not involve human or animal subjects; therefore, no ethical clearance was required. The study was based on bioinformatics analysis.

Author contributions

NW and TA conceived and designed the study, supervised the research, and revised and finalized the manuscript. FF collected basic data. KH conducted the research, analysed the data, and wrote the manuscript. MR designed the study and MA revised and finalized the manuscript.

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