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Original Communication

Cloning and protein structure prediction of DBL2β-PfEMP1 recombinant protein from Indonesian *Plasmodium falciparum* isolate

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ABSTRACT

The DBL2β-PfEMP1 is an adhesive domain of *Plasmodium falciparum*, which is important for malaria pathogenesis. In this study, the DBL2β-PfEMP1 from the Indonesian isolate of *Plasmodium falciparum* was cloned and the protein structure prediction of the DBL2β recombinant protein as well as its ligand binding sites was carried out. The DBL2β recombinant protein consists of 1674 nucleotides which are translated into 558 amino acids. Analysis using Expasy ProtParam tool showed that the protein had a MW of 64.69 kDa with an isoelectric point of 8.82. It had 83 negatively charged residues (Asp + Glu) and 98 positively charged residues (Arg + Lys). It was classified as an unstable protein because it had an instability index of 40.01. Protein structure prediction of the DBL2β recombinant protein and its binding sites was carried out using the I-TASSER program. It showed that the DBL2β recombinant protein had the highest significant alignment with the DBLβ domain of PF11_0521 PfEMP1, which is bound to the human ICAM-1, but the protein had the closest structural similarity with the of EBA-175 Region II (RII) of *P. falciparum*, where the protein functions as the cell invasion molecule. The highest C-score of ligand-binding site was 0.10 for the PEPTIDE ligand (GLN, LEU, ASP, PHE, GLU, ASP, VAL, TRP, ASN, SER, SER, TYR), and the ligand-binding site residues were at 84, 87, 88, 91, 92, 95, 99, 196, 199, 200, 203, 206. It is likely that the DBL2β recombinant protein has the major function as an adhesion molecule for invasion to the host. Further studies on its role in *in vivo* models are needed to develop a definite conclusion.

KEYWORDS: *Plasmodium falciparum*, DBL2β, PfEMP-1, Indonesia, protein.

INTRODUCTION

Plasmodium falciparum is the most deadly malaria agent among *Plasmodium spp*. The severity of malaria falciparum is due to the mechanism called cytoadherence i.e. the capacity of infected erythrocytes to adhere to vascular endothelium and other host cells through several receptors. This mechanism may result in the obstruction of microcirculation leading to poor perfusion of host tissues, hypoxia, and dysfunction of organs resulting in multiple organs failure [1]. One of the important proteins which is responsible for this mechanism is *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1).

PfEMP1 is a polymorphic protein and secreted during the erythrocytic cycle. The protein is transported from the parasite, which is located inside the erythrocyte, to the surface of the infected erythrocyte. It is deposited on the surface of erythrocyte in the structure known as the knob [2]. PfEMP1 is encoded by the *var* gene family which

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consists of approximately 60 variable genes per haploid genome of the parasite. It contains an extra-cellular and an intra-cellular part. The extra-cellular part contains the N-terminal segment (NTS) followed by two distinct binding domains: Duffy binding-like (DBL) and Cysteine-rich inter-domain regions (CIDR) [3]. The DBL domain is classified into six types based on the consensus motifs α, β, γ, δ, ε, and x. The CIDR domain consists of semi-conserved stretches and is classified into three different types: α, β and γ. The domain architecture is variable, where the sequence, number, location and type of domains differ significantly [4, 5].

Each PfEMP1 binding domain will bind to specific host cell receptor. Several studies have reported that DBL2β domain mediates the binding to ICAM-1 in several *P. falciparum* isolates [6]. In this study, we cloned the DBL2β domain of *Plasmodium falciparum*, which was isolated from an Indonesian malaria patient and carried out the protein structure prediction of the DBL2β recombinant protein as well as its ligand-binding sites.

MATERIALS AND METHODS

Malaria sample and ethical approval

Blood was collected from a severe malaria patient from Primary health care in Jember District, Indonesia. The patient was diagnosed with severe malaria with severe anaemia as a complication. The study was approved by the Ethical Committee of Research of Faculty of Medicine, University of Jember.

Cloning and structural analysis

The DBL2β domain was amplified by polymerase chain reaction (PCR) using specific primers from the DNA sample of the malaria patient. The PCR product was cloned into pJET1 cloning vector. The clone was confirmed by sequencing. The sequences were analysed using Expasy translation tool and the amino acid sequence was further analysed by ProtParam analysis [7]. The structure of the recombinant protein based on amino acid sequences was predicted using the I-TASSER program [8, 9, 10].

RESULTS AND DISCUSSION

The DBL2β recombinant clone was sequenced, and it consisted of 1674 nucleotides. Translation by Expasy translate tool resulted in 558 amino acids. Analysis of amino acid sequences using Expasy ProtParam tool showed that the protein had a MW of 64.69 kDa with an isoelectric point of 8.82. It had 83 negatively charged residues (Asp + Glu) and 98 positively charged residues (Arg + Lys). DBL2β recombinant protein was classified as an unstable protein because it had an instability index of 40.01.

Structure prediction of the protein using I-TASSER program is presented in Fig. 1. Five models were created based on the pair-wise structure similarity and grouping into cluster; the five models correspond to the five largest structure clusters.

Further analysis was conducted using Template Modeling (TM)-align program; it is an algorithm for sequence-independent protein structure comparisons. It generates optimized residue-to-residue alignment based on structural similarity using dynamic programming iterations and is presented as a TM-score, which has a value of 0-1, where 1 indicates the perfect match between two structures. The analysis showed that the DBL2β recombinant protein had a TM-score of 0.667, which is similar to that of the crystal structure of EBA-175 Region II (RII) of *Plasmodium falciparum* (DOI: 10.2210/pdb1ZRO/pdb) and a TM score of 0.657, which is similar to that of the crystal structure of *Plasmodium falciparum* Erythrocyte Binding Antigen 140 (PfEBA-140/BAEBL) (DOI: 10.2210/pdb4GF2/pdb), meaning that the DBL2β recombinant protein had a close structural similarity to those proteins, where the protein functions as the cell adhesion and invasion molecule. The high TM-scores of those proteins indicated a close structural similarity and further implicated a similar function of the DBL2β recombinant protein with those proteins.

I-TASSER program also measured the significance of alignment, presented as Z-score, where the Z-score >1 means a good alignment. The DBL2β recombinant protein showed the percentage sequence identity of 0.53 and the Z-score of 2.49, which are similar to those of the DBLβ domain of PF11_0521 PfEMP1 which is bound to human ICAM-1 (DOI: 10.2210/pdb5MZA/pdb); the protein has the cell invasion function. The Z-score of 2.49 indicated a good alignment of those proteins. Other proteins which have similar alignments are the

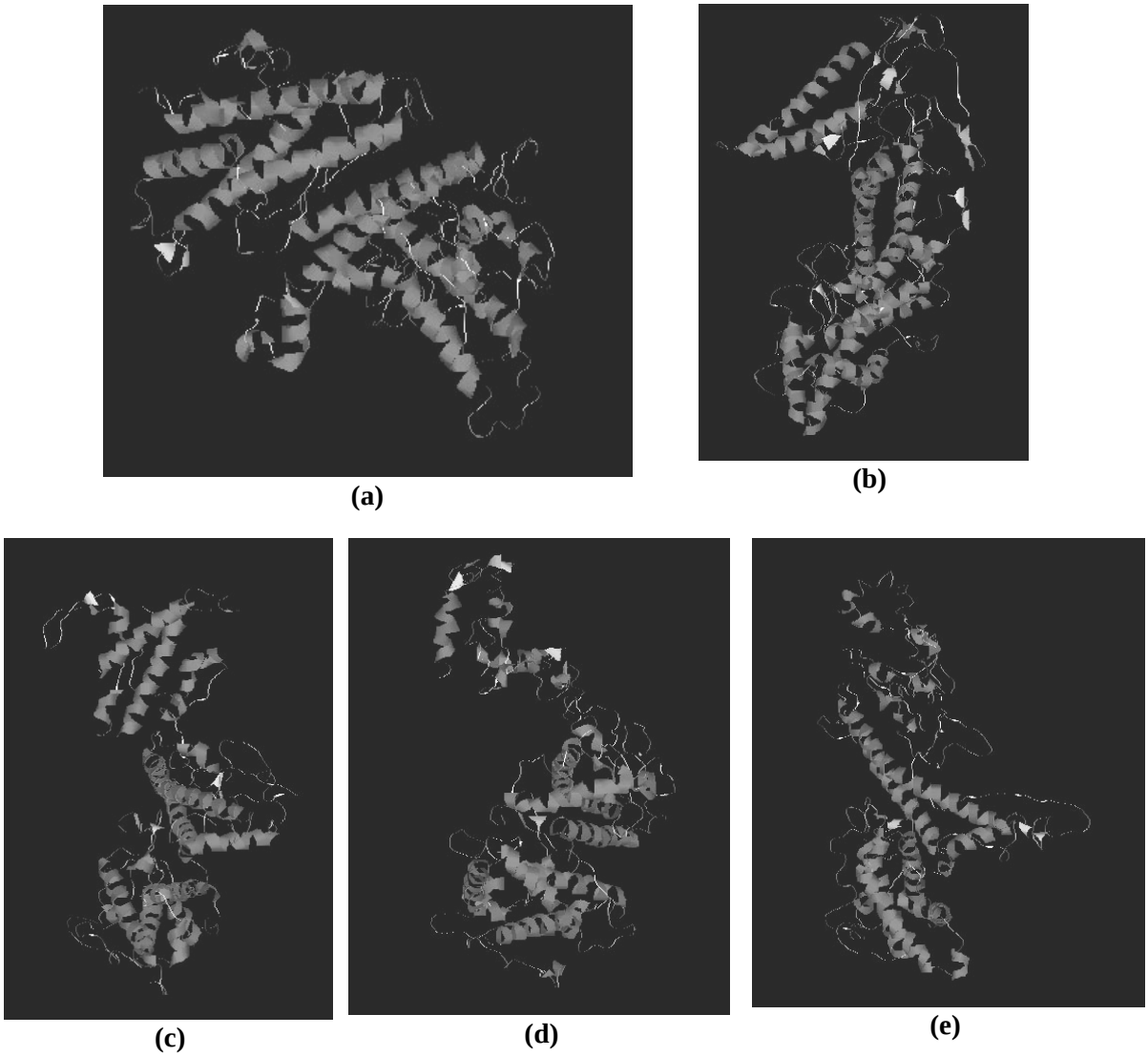

Fig. 1. Structure prediction of DBL2β recombinant protein. Structure was predicted using the I-TASSER program. C-score (Confident score) was calculated based on the significance in the threading template alignments and the convergence parameters of the structure assembly simulation at a range of (-5 - 2); the higher the C-score the higher the confidence of predicted model. (**a**) model 1: C-score -0.73; (**b**) model 2: C-score -0.20; (**c**) model 3: C-score -1.14; (**d**) model 4: C-score -3.10; (**e**) model 5: C-score -3.00.

structure of N-terminal NTS-DBL1-alpha and CIDR-gamma double domain of the PfEMP1 protein from *P. falciparum* varO strain (DOI: 10.2210/pdb2YK0/pdb), the *Plasmodium falciparum* Erythrocyte Binding Antigen 140 (PfEBA-140/BAEBL), and the EBA-175 Region II (RII) (DOI: 10.2210/pdb1ZRL/pdb). All proteins have the function of either cell adhesion or cell invasion. As mentioned, the high similarity in alignment of the DBL2β recombinant protein to those proteins indicated the similar function of the DBL2β recombinant protein as those proteins, either cell adhesion or cell invasion.

Gene ontology was analyzed by evaluating global and local similarity of the proteins and presented as the C-score^{GO}, where the value is 0-1. The DBL2β recombinant protein had the C-score^{GO} of 0.36, which is similar to that of the crystal structure of EBA-175 Region II (RII) of *P. falciparum* (DOI: 10.2210/pdb1ZRO/pdb), implicating an adequate confident prediction.

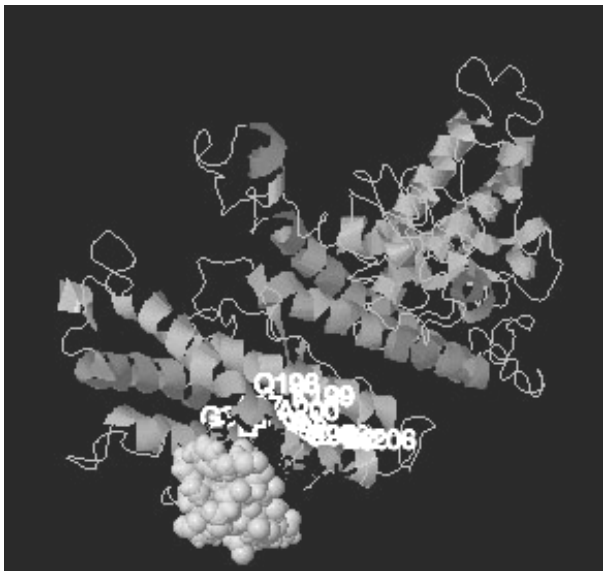

Fig. 2. Prediction of ligand-binding site of DBL2β recombinant protein, conducted by I-TASSER program. The binding site residues were at 84, 87, 88, 91, 92, 95, 99, 196, 199, 200, 203, 206.

Prediction of ligand-binding site showed that the highest C-score of ligand-binding site (0.15) was for the PEPTIDE ligand (GLN, LEU, ASP, PHE, GLU, ASP, VAL, TRP, ASN, SER, SER, TYR) and the ligand binding site residues were at 84, 87, 88, 91, 92, 95, 99, 196, 199, 200, 203, 206, as presented in Fig. 2.

The DBL2β recombinant protein from the Indonesian isolate of *Plasmodium falciparum* showed high structural similarity to several proteins that function as an invasion and adhesion molecule of *Plasmodium falciparum.* The high structural similarity implicated a similar function as either cell adhesion or cell invasion molecule. Previous studies have shown that the DBL2β domain of PfEMP1 binds to ICAM-1 and is associated with the development of cerebral malaria [11].

CONCLUSION

The DBL2β recombinant protein from Indonesian *P. falciparum* isolate showed high structural similarity to several *P. falciparum* proteins globally, which play a role in cell adhesion as well in cell invasion mechanisms. Further studies on its role in severe pathogenesis in *in vivo* models are needed to develop definite conclusion.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest.

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