THREE-DIMENSIONAL STRUCTURE BY HOMOLOGY MODELING AND FUNCTIONAL CHARATERIZATION OF FALCIPAIN-3: HEMOGLOBINASE FROM THE MALARIAL PARASITE *Plasmodium falciparum*

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Abstract: Falcipain-3 (FP3), the major cysteine proteinase of the human malarial parasite *Plasmodium falciparum*, is a hemoglobinase and promising drug target. The objective of this study is to investigate three-dimensional structure and functional characterization of the interactions between Falcipain-3 and hemoglobin complexes in *silico* modeling. This research predicted new three-dimensional-structure of the FP3 by using homology modeling module of Insight II. The interactions between the FP3 and the hemoglobin were analyzed through protein-protein docking followed by molecular dynamics simulations (MD). The result found that the sequence of the FP3 was closely similar to Falcipain-2 (1YVB) about 64%. The structure indicated that N-terminal extension of the FP3 was longer than FP2. The first residue (Thr1) was pointed inward and formed the hydrogen bond into the active site of the enzyme. The catalytic dyad of the enzyme was Cys51 and His183. The active site of the FP3 was composed of the amino acid about 15 residues that involved for binding with hemoglobin. The docking energy of protein-protein interactions indicated that the FP3 could be bound the hemoglobin at both α and β-chain of hemoglobin. The binding mode showed the side chain of catalytic dyad of the enzyme oriented their side chains pointed to heme molecule. At the equilibrium of the MD simulation (2ns), the nose chain of FP3 (residues 1-25) was contributed for the enzyme strong binding to the hemoglobin and the arm chain (residues 194-207) showed highly flexible during simulation.

Introduction

Various potential biochemical targets have been proposed and are being pursued for the de novo design of novel antimalarials [1]. Among these targets are proteases that hydrolyze hemoglobin and are known to play vital roles at various stages of the parasite life cycle [2]. The cysteine proteinases are the major reported hemoglobinases present in the food vacuole. In *P. falciparum,* three papain-like cysteine proteinases thus Falcipain-1, Falciapin-2 and Falcipain-3 have been identified and characterized. Among them, the Falcipain-3 is to be more important due to it appears to cleave native hemoglobin about twice as rapidly as the former. However, the crystal structure of the Falcipain-3 has no report in the Protein Data Bank. Here, this research proposed the three dimensional structure of Falcipain-3 obtained from Homology modeling. The resulting model is suitable for further study of structure based drug design against malaria. The docking studies including molecular dynamics simulation are also provided insight into the possible binding modes and interactions of hemoglobin with the enzyme.

Results and Discussion

Homology modeling: A homology model for Falcipain-3 was derived based on the multiple sequence alignment of the Falcipain-3 sequence which obtained from SWISS-PROT (GenBank accession no.Q9NAW4) with homologs as shown in Figure 1. The average sequence homology of Falcipain-3 with the five homologs was 35%. However, the sequence of the Falcipain-3 was closely similar to the Falcipain-2 about 62.5%. The 3D structure of the Falcipain-3 was shown in Figure 2. The structure divided into two domains, L and R. The extra motif, N-terminal, of the Falcipain-3 had longer than Falcipain-2. The structure composed of five helices, six stands and six turns. The active site of Falcipain-3 was located between domain L and R and its composed of amino acid about 15 residues. The catalytic dyad of the Falcipain-3 presented to the Cys51 and His183.

Docking: The model of Falcipain-3-hemoglobin complexed was shown in Figure 3. The model showed that, Falcipain-3 was bound against hemoglobin at chain A and it located nearby the heme ring molecule. The amino acids of Facipain-3 involved for binding located within 5 Å of catalytic dyad such as Gln45, Gly49, Cys89, Tyr90, Tyr93, Ile94, Ser158, Pro181, Glu243, Ala160, Ala161, Ser162, Ala166, His183, Trp215, Lys85, Asn86, Asn87, Gly91 and Gly92. These residues could divide into 4 subsites, S1, S2, S3 and S1′. The amino acids in each subsite were shown and list in Figure 4 and Table 1, respectively. The interactions between Falcipain-3 and hemoglobin were shown that, the N-terminal of the enzyme contributed for binding. Thr1 of the enzyme located nearby Cys51 and hemoglobin with distance 3.4 and 3.5 Å, respectively.

MD simulation: In order to MD simulation, the complex model was run to equilibrium about 2 ns in the water system. The trajectory extracted from the complex coordinates at 2 ns and the results found that Falcipain-3 and hemoglobin were bound together with 6 hydrogen bonds and hydrophobic interaction (Figure 5).

In addition, our research was also docking the artemisinin into the active site of Falcipain-3. The artemisinin (also called "qing hao") is a drug used to treat multi-drug resistant strains of *falciparum* malaria. We postulated that artemisinin can be inactivates against Falcipain-3. The result of the interaction was shown in Figure 6. The results shown that artemisinin could be fit bound in the active site of Falcipain-3. The binding energy obtained from docking was -6.80 kcal/mol. The artemisinin reacted with the enzyme with 3 hydrogen bonds. His183 and Asn182 were generated bonds with artemisinin about 2.8 and 3.1 Å, respectively.

Computational methods

The experiments were performing on a silicon graphic O2 workstation with homology module of InsightII. The protein-protein interaction of Falcipain-3 and hemoglobin studies were investigated with docking module of Accelrys Discovery Studio 2.1 and the molecular dynamics (MD) simulations were performed through GROMACS 3.3.1.

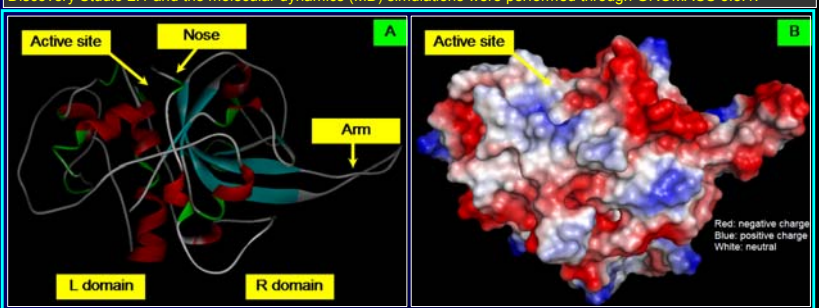

Figure 2 A: Structure of Falcipain-3 model which obtained from homology modeling. The structure has shown the helix, strands, loop and turn in red, blue, gray and green, respectively. Important motifs are nose and arm region. In particular of nose, the first 26 residues, it located nearby the active site of Falcipain-3. B: Electrostatic surface was shown the region where presenting the negative, positive and neutral which were colored in red, blue and white, respectively. The active site of the Falcipain-3 is a pocket located between domain L and R. The arm motif presented the negative region and it fully exposed to solvent.

Table 1 List of important amino acid residues and subsites identified that lining the binding pockets of the Falcipain-3

Subsite	Amino acid residues
S1	Gln45, Gly49, Cys89, Tyr90
S2	Tyr93, Ile94, Ser158, Pro181, Glu243
S3	Lys85, Asn86, Asn87, Gly91, Gly92
S1′	Ala160, Ala161, Ser162, Ala166, His183, Trp215

Figure 1 Multiple comparisons of amino acid sequence of the papain-like cysteine proteinase. The important regions which necessary for binding with the hemoglobin are located in the pink box. The similar residues were shaded in red. The sequence of the Falcipain-2 is similar to the Falcipain-3 about 62.5%.

Figure 5 Close views of interactions between Falcipain-3 and hemoglobin. These interactions obtained from MD simulation at 2 ns. The structure of hemoglobin and heme were shown in yellow ribbon and green stick, respectively. The 6 hydrogen bonds were generated and shown in green dashed line.

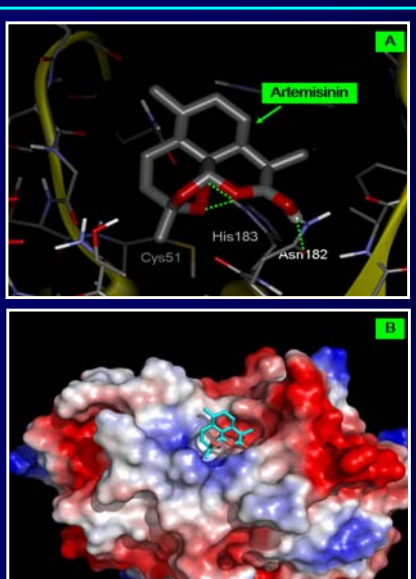

Figure 6 Left(A): Binding mode of artemisinin within the active site of the Falcipain-3. Falcipain-3 and artemisinin were shown in yellow ribbon and stick, respectively. Three hydrogen bonds of their interactions were presented in green dashed line. Left(B): Electrostatic surface of the Falcipain-3 presented the artemisinin was fit bound in the cleft of the active site.

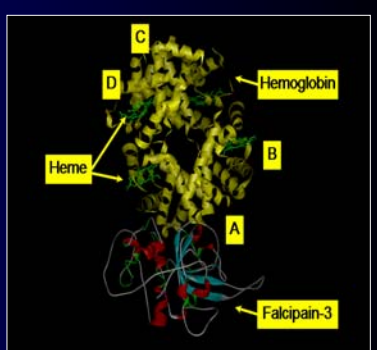

Figure 3 Structure of Falcipain-3-Hemoglobin complexed were obtained from Protein-Protein docking method.

Figure 4 The active site of Falcipain-3. The structure of the enzyme was shown in magenta ribbon and the amino acid residues surrounded within 5 Å of catalytic dyad (Cys51 and His183) shown in atom colored stick.

Conclusion

Our research was proposed the three dimensional structure of Falcipain-3 and also presented the interaction between the enzyme with hemoglobin and artemisinin. The structure of Falcipain-3 is closely similar to Falcipain-2. The N-terminal of the enzyme is important for binding with hemoglobin. Our complex model is suitable for explain the binding mode of hemoglobinase. Importantly, we also conclude that, the artermisinin can fit bind within the active site of 3. It can be use to further drug development against malaria.

Acknowledgements

This work was supported by the Thailand Research Fund, the Royal Golden Jubilee Ph.D. program (RGJ) grant to K. P., and Mr. Patarapon Juntrapon, Thai Equipment Research Co., Ltd. for providing the Accelrys Discovery Studio 2.1 program.

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