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In silico Design of Drugs and Vaccines for Dengue Disease

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ABSTRACT

The aim of the study is to conduct modeling of NS3 protease (pro) enzyme and E DENV. These two approach have different means. The first is developed for producing drugs and the second is for producing vaccines. Crystal structures of the related NS3pro from Hepatitis C Virus (HCV) have been used successfully as a model template in drug discovery, in order to provide some insight into the structure function of the protease and the substrate and cofactors binding motif and thus facilitate substrate-based inhibitor design of the dengue 2 virus NS3. The objective of E DENV *in silico* research is to design dengue virus vaccines with *in silico* method, using E DENV-2 and E DENV-3 protein as their backbones, which could give immune response toward four dengue virus serotype. The *in silico* NS3pro research in this review shown, that the development of Dengue drug design will rely upon its structure and reactivity. Our vaccine design are utilized based on different algorithms. We were using Artificial Neural Network (ANN) and Hidden Markov Model (HMM) algorithms. The complexity of virus-lead compound and virus-immune system interaction need to be computed with large computational power. Henceforth, both approach could be utilized for developing real drugs and vaccines at the wet laboratory.

Key words: Dengue, E DENV, NS3, protease, *in silico*

INTRODUCTION

Dengue fever is an acute febrile viral disease characterized by sudden onset, fever of 3-5 days, intense headache, myalgia, anthralgic retro-orbital pain, anorexia, GI disturbances and rash. Dengue viruses are member of flavivirus family. They included four serotypes of 1, 2, 3 and 4 (Dengue- 1, -2, -3 and -4). These viruses are also responsible for Dengue Hemorrhagic Fever (DHF). The viruses are transmitted to human by the bite of infective mosquitoes, mainly Aedes aegypti. The incubation period is 4-7 days (range 3-14 days). This disease is now endemic in most tropical countries. DHF is characterized by increased vascular permeability, hypovolaemia and abnormal blood clotting mechanisms (Chatuverdi *et al*., 2005).

Dengue Fever (DF), with its severe manifestations such as DHF and Dengue Shock Syndrome (DSS), has emerged as a major public health problem of international concern. The geographical distribution has greatly expanded over the last 30 years, because of increased potential for breeding of Aedes aegypti, the vector species. This has been prompted by demographic explosion, rapid growth of urban centers with a strain on public services, such as potable water. Breeding of Aedes aegypti was expanding rapidly due to rainwater storing in diverse types of containers (WHO, 2007).

About 100 countries are endemic for DHF and about 40% of the world population (2.5 billion people) is at risk in the tropics and sub-tropics region. Over 50 million DF infections with about 400,000 cases of DHF are reported annually which is a leading cause of childhood mortality in several Asian countries (Guglani and Kabra, 2005).

According to Indonesian Department of Health fact sheet, DF is on the rank 8 of 10 infection diseases, which are considered important in the budget priority and political commitment. All of the dengue virus serotype (DENV) are endemic in many of the big cities and occurred at yearly basis IVI team, 2007.

The vaccine of DF is not yet available. The overwhelming obstacles of the dengue vaccine development are the unknown pathogenesis of dengue virus in human host and the difficulty of virus growth in the culture medium. The animal testing was done for immunogenic and disease prevention purpose only (Elgert, 1996).

An attenuated vaccine is under clinical trial on human subject till date. The vaccine was developed by Mahidol University at Bangkok and Walter Reed Army Institute of Research at the United States. There are risks that could occur by misusing attenuated vaccine. If the virus in the vaccine is not attenuated enough, the vaccine will attack the human host like the DENV infection. Moreover, if the vaccine contains over attenuated virus, the vaccine would not induce the body immune response (Halstead and Deen, 2002).

The modern engineered vaccine for DENV infection is necessary; in order to overcome the problems of attenuated vaccine. One of the engineered vaccine types is protein/peptide vaccine. Protein/peptide vaccine consists of peptide sequence which derived from the small part of pathogenic protein. There are antigenic determinant in certain part of the vaccine peptide sequences (Gancalves *et al*., 2004; Kols and Sehrris, 2000; Thomson *et al*., 1998).

Recently, at University of Dhaka, Dhaka, in 2008, it has also been established that active NS3 serine protease is an essential requirement for maturation of the active Dengue virus type 3. Another study also confers the involvement of a NS3 serine protease (pro) in Dengue virus type 2. Crystal structures of the related NS3pro from Hepatitis C Virus (HCV) have been used successfully as a model template in drug discovery and a structure of DEN NS3pro lacking the NS2B (1BEF) cofactor is available. However, the residues and other structural elements that play a role in the enzyme-mediated maturation process of DENV by NS3 have yet to be definitely assigned. Identification of the binding site and the actual environment around the active site pocket are still open to questions and are of fundamental importance in understanding both the viral maturation mechanism and the significance of inhibitors nature, which may be directly responsible for future drug designing.

As a key step toward elucidating the functions of DENV NS3 and in particular for a better understanding of the active site pocket of the enzyme, the 3D structure of DEN2 NS3 serine protease to locate its catalytic residues has been modelled. Comparative modeling of sequences and structures which related NS3 serine proteases have also helped to predict the electrostatic potential distribution within and around the active site pocket of NS3 (Jesmin and Sharmin, 2006).

Join team from University of Queensland and Herston Royal Children Hospital at 1999 has made a research about NS3 as well. Crystal structures of serine proteases encoded by members of both the Togaviridae and the Flaviviridae have been reported. However, only the crystal structure of the NS3 protease of Hepatitis C Virus (HCV) incorporates a virus-encoded cofactor, as found in the flaviviruses. The basic features of that protease are two six-stranded **β**-barrel domains, characteristic of the chymotrypsin-like fold, that are separated by a linker region. In addition, there

is an intimately bound structural peptide encoded by NS4A that functions as the cofactor-equivalent of NS2B in the flaviviruses. The potential of using these structural data to extrapolate to proteases encoded by other members of the flaviviridae was recently addressed. This research tried to make homology modeling of dengue 2 virus NS3 protease using HCV NS3 protease as a template, in order to provide some insight into the structure and function of the protease and the substrate and cofactors binding motif and thus facilitate substrate-based inhibitor design of the dengue 2 virus NS3 (Brinkworth *et al*., 1999).

Othman *et al*. (2008) from University of Malaya, Sunway University college and Univeristy of Hawaii have reported automated docking studies performed on the compounds which exhibited noncompetitive inhibitory activities toward NS2B-NS3 of Dengue virus type 2, using AutoDock 3.0.5 and Glide24 software. The subjects of this study are the flavanones: pinostrobin, pinocembrin and alpinetin and their chalcone derivatives: pinostrobin chalcone, pinocembrin chalcone and cardamonin, respectively. The aim of their study is to understand the interactions involved in the binding of these compounds (referred to as ligands hereafter) to NS2B-NS3 of Dengue virus type 2 via computational docking methods and to gain insights into the experimental inhibition pattern. It is hoped that information from this study will provide further understanding of the mechanism of inhibition of Dengue virus type 2 protease and enable the design of antiviral drugs which inhibit dengue virus replication (Othman *et al*., 2008).

The multiple alignment study of dengue virus tetravalent vaccine design has been done by our group at 2009 in Jakarta. The multiple alignments that were done with 102 dengue virus intra serotype of the four DENV serotype concluded high similarity of E protein among each of DENV intra serotype. Although, mutation occurred on E protein, the antibody can recognize all DENV intra serotype as one dengue virus serotype. The general objective of this research is to design dengue virus vaccines with *in silico* method, using E DENV-2 and E DENV-3 protein as their backbones, which could give immune response toward four dengue virus serotype (tetravalent). The specific objective of this research is to design and predict the tertiary structure of dengue virus tetravalent vaccines by homology modeling method and determine which vaccines that have the best functionality according to its tertiary structure (Tambunan *et al*., 2009).

***In silico* study of DEN2-NS3 protease:** Modelling the 3D active site pocket of DEN2-NS3 serine protease to have a better understanding of the structure-function mechanism of NS3 serine protease of DEN2 serotype. The 3D structure of DEN2-NS3 protein was modelled and analysed using Swiss-Pdb Viewer. The crystal structure data (PDB id: 1BEF) along with the Multiple Sequence Alignment (MSA) and Ramachandran's plot data were used to generate a 3D model of DEN2-NS3. Hydrogen bonds were built within the key residues of the 3D active site pocket. This enables the DEN2-NS3 structure to be compared with other known serine proteases. The NS3 protease showed a chymotrypsin-like fold with β-barrels, formed by six β-strands, with the catalytic triad (His51-Asp75-Ser135) located at a cleft (Fig. 1a, b) (Jesmin and Sharmin, 2006).

The successfull modeling study of NS3 protease active site will eventually shed light to the structure and reactivity of the drug candidate (lead compound).

Modeling study of the NS2B cofactor and its substrate interactions with DEN2pro: The substrate specificity of Dengue type 2 protease at the P1 position is similar to that of trypsin in recognizing basic lysine or arginine residues. This is defined for trypsin by an acidic Asp residue at position 189, six residues before the catalytic Ser195, which forms a salt bridge with the lysine or arginine (Fig. 2a-c). Significantly, DEN2pro has an equivalent Asp at position 129, six residues

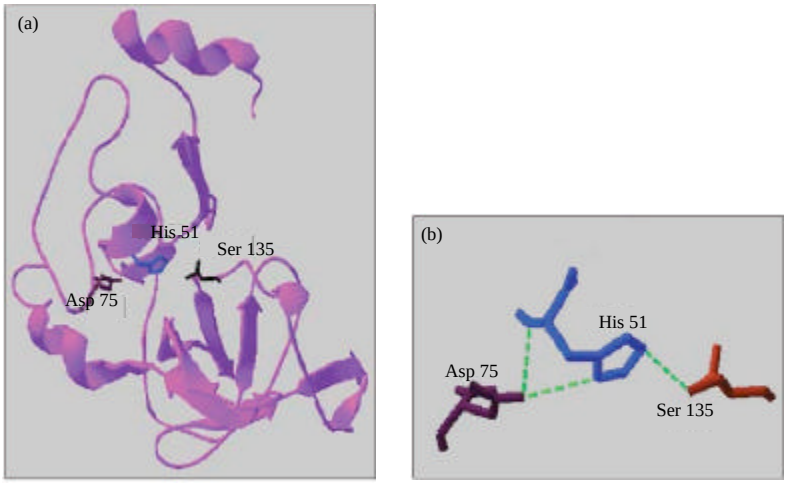

Fig. 1: Proposed model of the active site pocket of DEN2-NS3. (a) Cartoon diagram of the proposed model. (b) Key active site residues. This finding is in agreement with a recent study of NS3 protease of West Nile virus (WNV) where similar structural patterns have also been reported

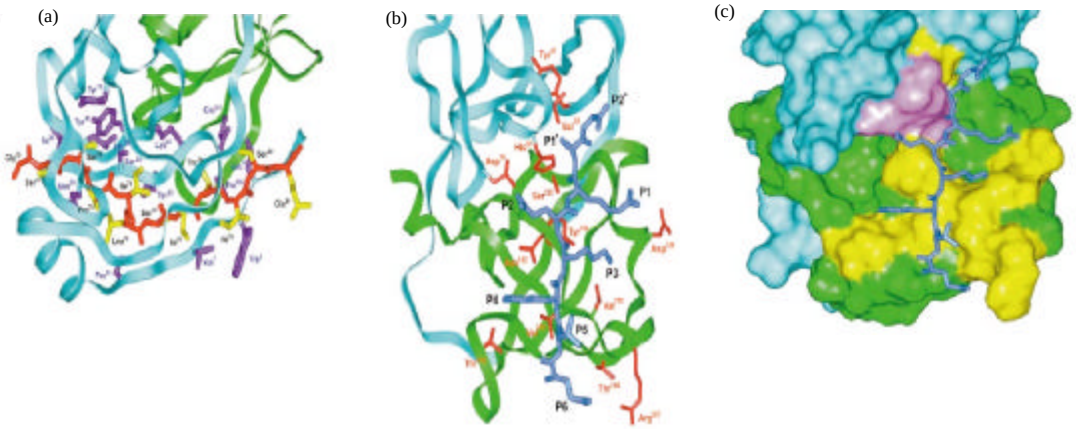

Fig. 2: (a-c) Structural models of the NS2B cofactor and substrate interactions with DEN2pro. The DEN2pro backbone is represented in cartoon form with the side-chains of likely contact residues in stick form in dark blue (a) or red (b). Domain 1 of DEN2pro is in light blue, with domain 2 in green. (a) Cofactor interactions. The cofactor is represented in stick form (in red) with residue side-chains in yellow. Individual residues are identified (cofactor residues in black and DEN2pro residues in blue). (b) Substrate interactions. The substrate (comprising residues surrounding the NS2B/NS3 cleavage site; P6-EVKKQRAG-P2h) is represented in stick form and colored blue. Individual residues are identified (substrate residues in black and DEN2pro residues in red). (c) DEN2pro surface plot with substrate as a 3D stick model in blue. DEN2pro contact residues are highlighted in yellow and members of the catalytic triad in purple

before the catalytic Ser135, that is highly conserved in all flavivirus NS3 sequences determined to date. Based on observations with the HCVpro crystal structure, it is suggested that this Asp residue may be located at the bottom of the P1 pocket for the flavivirus NS3, as is the case for thrombin. The model suggests, however, that Asp129 lies at the end of a P1-binding trough (Fig. 2c). It is also suggested that these results could be explained by the substituted amino acids still retaining contact with the substrate via a water molecule. Alternatively, given the specificity of flavivirus proteases for two basic residues at the P1 and P2 positions, the loss of one contact may not be sufficient to eliminate activity. It is interesting to note in this context that the catalytic triad member Asp75 is indeed within hydrogen-bond distance of P2. The interaction of P2 with Asp75 would provide additional structural stability for the enzyme, given the link this bond forms between the two protein domains (Fig. 2b). During energy minimization of the enzyme-cofactor complex model, it was found that in the absence of substrate the two domains of the enzyme and hence, the catalytic triad residues, moved apart (Brinkworth *et al*., 1999).

This study is spesifically aimed at protease activity of the enzyme. The thorough study of the reactivity between the amino acid residue of the catalytic site and the substrate will be crucial for the effective binding of the drug lead compound.

Docking of non competitive inhibitor with DENV2 protease: Figure 3a-d show the orientations of cardamonin, alpinetin, pinocembrin and pinocembrin chalcone in the binding site of the protease, respectively. Except for pinocembrin chalcone, these ligands experienced H-bond and van der Waals interactions with almost the same surrounding residues. The common residues forming H-bond with the three ligands were Leu149 and Asn152. In addition, pinocembrin formed

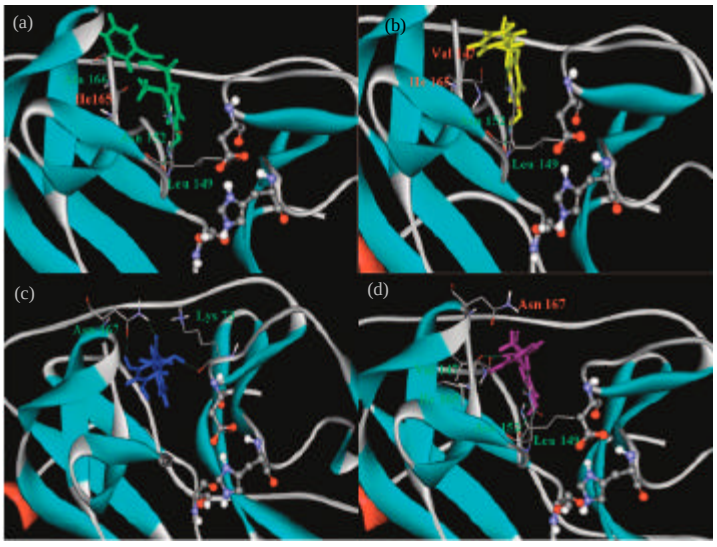

Fig. 3: Models of ligands bound to the protease (cartoon) at the binding sites. From left to right, The ligands shown are (a) cardamonin (green), (b) alpinetin (yellow), (c) pinocembrin chalcone (blue) and (d) pinocembrin (purple). Residues interacting with the ligands are shown as sticks. Catalytic triads are shown as balls and sticks. Residues labeled in green interact with the ligands via H-bonds, while those in red interact via van der Waals contacts

an additional H-bond with Val147 and Ile165. In terms of the van der Waals interactions, cardamonin interacted with Ile165, while alpinetin interacted with Val147 and Ile165 and pinocembrin with Asn167. Pinocembrin chalcone, however, did not demonstrate van der Waals interaction with any of the surrounding residues but interacted with Lys73 and Asn167 via H-bond (Othman *et al*., 2008).

Overall, the *in silico* NS3pro research in this review shown, that the development of Dengue drug design will rely upon its structure and reactivity. Whether the existence of the H-bond and Van Der Walls bond between the residue and substrate have direct correlation within the lead compound reactivity, it should be verified in the wet laboratory. The chemical modeling of ligand-protein interaction is very crucial for the determination of lead compound reactivity (Goodsell *et al*., 1995).

***In silico* study of E DENV protein:** This dengue virus tetravalent vaccine design was done by *in silico* method. The method was using E DENV-2 protein and DENV-3 as their backbones. The reason for choosing E DENV protein was because of its function on viral attachment at the host cell surface and for facilitating immune response at the host cell. The E DENV-2 and E DENV-3 proteins were chosen as the backbones because of their high prevalence in the South East Asian region (8,19). Vaccine design was accomplished by using homology modeling method. This method needs three dimensional protein molecule structure in the PDB format. PDB files could be downloaded from Protein Data Bank, which supervised by Research Collaboratory for Structural Biology in their websites http://www.rscb.org/pdb and could be accessed freely by internet access. The E DENV-2 and DENV-3 files had been found (Tambunan *et al*., 2008).

The *in silico* dengue epitope study is very important for designing vaccine. The information are useful as an insight for our own developed *in silico* vaccine design. However, it should be taken into account, that every computational algorithms for epitope prediction, will produce different information sets. The vaccine design for each algorithms should be different with each other (Fig. 4, 5).

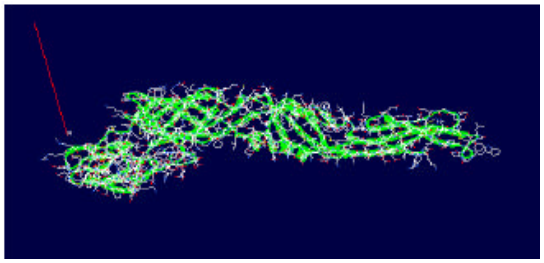

Fig. 4: Our *in silico* ANN1 vaccine design

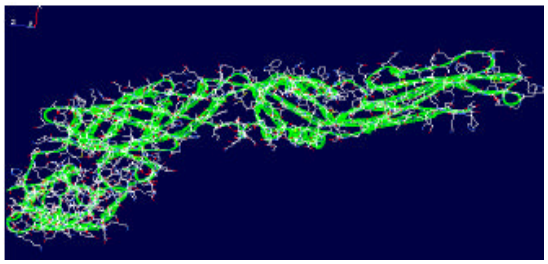

Fig. 5: Our *in silico* HMM1 vaccine design

Our vaccine design are utilized based on different algorithms. We were using Artificial Neural Network (ANN) and Hidden Markov Model (HMM) algorithms. However, the efectiveness of the different algorithms must be proven in the wet laboratory experiment.

DISCUSSION

Falgout *et al.* (1991) from National Institute of Allergy and Infectious Diseases, Bethseda, Maryland, in 1991, have done wet experiment about NS2/NS3 protein. By using recombinant vaccinia virus expression system, they found that A polyprotein composed of NS2A, NS2B and the N-terminal 184 amino acids of NS3 was cleaved at the NS2A/NS2B and NS2B/NS3 junctions, whereas a similar polyprotein containing only the first 77 amino acids of NS3 was not cleaved. Falgout *et al.* (1991) research has gave insight on NS3 protein function.

The *in silico* researches of NS3 protease presented in this articles are the direct continuation of the previous wet laboratory research. Falgout *et al.* (1991) did not make any modeling or important computational approach. Based on wet experiment, the *in silico* research were utilizing those data, to make a better structural and reactivity modeling.

Present research about E DENV are implementing important scientific approach, which presented in Novartis proceeding 2003. Here, we present the, red thread between them.

Brusic and Petroski had predicted the flow chart and necessary steps for developing modern vaccine, including the future for computational modeling. The acceptance of immunoinformatic techniques by clinical and research immunologists will need robuststandards of data quality, system integrity and properly validated immunoinformatic systems.

Faced with the expanding volume of information now available from genome databases, De Groot and William were suggesting for using to epitope mapping tools to screen vaccine candidates. Bioinformatics tools such as EpiMatrix and Conservatrix, which search for unique or multi-HLA-restricted (promiscuous) T cell epitopes and can ¢nd epitopes that are conserved across variant strains of the same pathogen, have accelerated the process of epitope mapping. When used together, these bioinformatics tools o¡er a significant advantage over traditional methods of vaccine design since high throughput screening and design is performed *in silico*. The research presented in this paper has utilized many methods or alogrithm which previously described in Novartis proceeding.

Basically, the research about E DENV protein are the implementation of methodology in Novartis immunoinformatics proceeding. Our labs has utilized the Brusic and Petroski approach and De Groot/William methodology. So, we could infer that there is a direct correlation between our research and the previous ones.

Earlierly, we don't conduct the wet lab experiment, because we are in the process of improving our modeling capabilities and on the process of searching the appropriate biomedic institute as our partner. Our group is in the process of approaching prominent biomedic institute in Indonesia, in order to realize our *in silico* design in the wet labs. We are planning in producing drugs and vaccine in the long terms.

Outlook on the future of dengue medication: The Different approaches of NS3pro and E DENV protein studies are designed for different kind of necessity. The NS3pro research are tend to be developed for drugs, while the E DENV protein are for vaccines. Because of its tendency as a computational biochemistry research, NS3pro modeling rely on specific method for computational chemistry, such as Monte Carlo Simulated Annealling. However, the E DENV research have

utilized a more common computer science algorithms, such as HMM and ANN. Although the methodology are different, these two different approaches, which based on computational chemistry and computer science, will always play critical role in biomedics research. These two different approaches have emphasized, that multi disciplinary research is the key for tackling with biomedical related subjects.

Moreover, the complicated protein structure elucidation of Dengue virus need huge computational power. The data generated from protein database will grow exponentially and data computation will became an issue. Luckily, the hardware and software of computer science are advancing rapidly as well. Sophisticated computer science algorithms, which are difficult to implement in the past due to the limited computational power, now are possible to be applied. The cost of the PC hardware is decreasing, while the performance is increasing. This situation will made hard core bioinformatics data computation possible to done on every laboratory.

However, there are some issues that needs to be relieved. First, The successful ligand binding *in silico* experiment doesn't correspond at it's delivery to the target receptor. It is widely known, that before reaching the target receptor, the ligand/lead compound must withstand the interference of various degrading or biotransforming enzyme. How to protect the lead compound before reaching its target, is an interesting research topics. A model simulation for protecting the ligand could be developed computationally. Second, the genetic variation of human could pose a problem for drug and vaccine design. The epitope prediction servers are already available, so the human immunogenic response has already taken into account in vaccine design. However, a more clever algorithms must be designed, in order to cover the whole genetic variation, especially concerning human immune system.

The *in silico* design of medications, whether for vaccine or drugs, will always play crucial roles in the biomedics research. Dengue is a fast mutating virus, so a new measure to tackle with dengue fever is necessary. Computational research on Dengue has generated a lot of information concerning its protein structure and reactivity. The next challenge would be to implement that information, into the wet laboratory biomedics research. However, the vast automatisation of the wet lab instruments will made the experiment possible.

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