Bioinformatic analysis of truncated envelope protein in C-terminal stem-anchor region: as strategies for increasing protein secretion

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Abstract. One type of Dengue vaccine candidate that has the potential to be developed is DNA vaccine based on the DENV envelope gene. Constraints in developing vaccines are low secretion of envelope proteins. One of the efforts made to increase the secretion of envelope protein is by deletion the stem / anchor area. The purpose of this analysis was to analysis the effect of cutting the stem / anchor region on character and structure of the DENV E protein. Analysis of composition, profile, location and structure of proteins was using www.expasy.org, TMHMM, and http://bioinf.cs.ucl.ac.uk/psipred. Deletion of the stem-anchor region was changes the general properties of the protein, as well as the protein topology. However, the mutations did not change the 3D structure of main protein domain.

1. Introduction
Dengue infection is one of endemic diseases in the tropics and subtropics regions. This disease infects 50-100 million sufferers each year worldwide. According to data from Litbangkes (2014), in Indonesia, dengue infection reached 101,218 cases in 2013 with a CFR (case fatality rate) of 0.7%.

Currently several DENV vaccine candidates are being developed with various approaches. Vaccine development using Recombinant DNA techniques is interesting to do because it is more efficient and safe. The importen things have been noticed in developing a vaccine using recombinant DNA technology is the selection of genes to be inserted into the vector plasmids. Immunity protection against DENV infection is generally mediated through neutralizing antibodies against structural envelope (E) glycoprotein virus. In addition, from several research reports it is known that in the E protein region there are many important epitope for viral neutralization process (Putri et al, 2015, Dewi et al 2014 and Ishikawa et al, 2014).

The challenge of developing a vaccine using E DENV protein-based recombinant DNA approach was the low of envelope proteins expression, resulting in low immunogenesis as well. One strategy to solve this problem is to modify the E DENV gene in the form of cutting of some amino acids in the C-terminal domain, especially in the transmembrane region of the stem-anchor (Raviprakash, 2000 and Hsieh, 2008). The mutations performed are not expected to alter the structure of E DENV protein. This bioinformatic analysis aims to analyze the differences in intact and removed EDENV proteins in the transmembrane region of the stem - anchor.
2. Methods and Materials
Protein bioinformatic analysis was performed on protein sequence E DENV-3 strain IDS 39/10. This sequence was a vaccine candidate developed in the laboratory of Microbiology FK-UI. The genes of E strain IDS 39/10 have been uploaded to genbank with accession number KF857536. E DENV protein sequence was obtained by translating the E DENV gene nucleic sequence using the BioEdit software. Mutation was done by removing (deletion) as many as 101 amino acids in the C-term of envelope protein (using only 80% protein envelope).

Initial information about the DENV envelope gene was obtained from the data base on www.ncbi.nlm.gov, www.uniprot.org and virus-mPloc software. Mutations are carried out referring to several literature studies. Analysis of composition, profile, location and structure of proteins was using www.expasy.org, TMHMM, and http://bioinf.cs.ucl.ac.uk/psipred

3. Result and Discussion
Deletion of 101 amino acids in the C-terminal envelope DENV-3 (stem / anchor region) changes all protein characters associated with the ProtParam program from the web www.expasy.org (Data not show). Topology analysis of DENV envelope proteins was carried out on wild type proteins and those mutated. The hydrophobicity of proteins was analyzed using the Hphob scale. / Kyte & Doolittle. The data in Fig. 1 shows that the stem / anchor region of wild type DENV-E protein was tends to be hydrophilic. Mutations carried out cause the protein to turn hydrophobic.

![Figure 1](image_url)

**Figure 1.** The DENV envelope protein topology based on hydrophobic properties using the Hphob scale. / Kyte & Doolittle. A; The wild type DENV E protein. B; DENV-E protein that was mutated

Transmembrane protein analysis was show the DENV E protein has two sites of a transmembrane protein. Deletion of the stem / anchor region can remove this transmembrane region (Fig.2).
Figure 2. DENV E protein helical transmembrane prediction using TMHMM program. A: The wild type DENV E protein. B: DENV E protein that was mutated.

Glycosylation motifs were analyzed using NetNGlyc software. The results of the analysis show that the DENV E protein has two glycosylation motifs. The mutations carried out did not change the glycosylation site on DENV E protein (Fig. 3). According to Amarilla et al. (2009), the motive of glycosylation in DENV E protein was found on the side of Asn-65 and Asn-153. Glycosylation was an important post-translation modification, and that affects the process of protein folding, localization, protein solubility, antigenicity, biological activity, and protein interactions with cells. Because mutations do not change the motive glycosylation of proteins, so it was expected that the structure and important functions of E DENV proteins also do not change.

Figure 3. The glycosylation motive of the DENV E protein. A: The wild type DENV E protein. B: DENV E protein that was mutated.
DENV E protein was the main target in the development of dengue vaccine. This protein has several important sites related to the function as candidate antigens. The amino acid sequencing at the beginning of the protein terminal was an important part in the protein cutting signal. The amino acid at position 98-109 was the part that will form a loop structure that was responsible for attaching the fusion of ectodomain proteins to the target membrane. This section was hydrophobic. DENV-3 E protein has 3 specific epitopes which will be recognized by 1B7 monoclonal antibodies, which were in the 50-57, 127-134 and 349-356 positions (Modis et al, 2005). The Pymol program was used to analyze the position of these sites on the DENV E protein surface (Fig. 4).

![DENV E protein diagram](image)

**Figure 4.** Modeling the position of important sites on the DENV E protein

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