Thesis title	Production of recombinant protein of dengue virus serotype 3 in *E. coli* system and purification by immobilized metal affinity chromatography
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ABSTRACT

Dengue hemorrhagic fever is one of the important diseases in tropical area including Thailand. The disease is caused by dengue virus transmitted through mosquitoes (*Aedes aegypi* and *Aedes albopictus*). Mature virions are composed of a single sense stranded RNA genome surrounded by nucleocapsid. The RNA genome encoded structural and non-structural proteins in the gene order :

5' C-preM-E-NS1-NS2a-NS2b-NS3-NS4a-NS4b-NS5 3'

The objective of this study is to produce a recombinant envelope (E) protein of dengue virus in *E.coli.* Genomic RNA of dengue virus serotype 3 strain H87 was extracted from the supernatant of dengue infected C6/36 cell culture. Amplification of E gene from extracted genomic RNA was performed by reverse-transcription and polymerase chain reaction (RT-PCR). The RT-PCR product, of 1264 nucleotides consisted of C terminal truncated envelope gene (den3E) and 5' and 3' flanking restriction sites for cloning purpose. The den3E gene contained 1,227 out of the full-length 1,479 nucleotides of envelope gene, encoding 409 amino acid residues, excluding 84 amino acid residues of the hydrophobic C terminus. The PCR product was digested by available restriction enzymes, *Bam*HI and *Eco*RI, and then cloned into commercial *E.coli* expression vector pTrcHisA. The *E.coli* clones containing recombinant plasmid, pTrcHisA/den3E, could produce the recombinant envelope protein with 6 histidine residues as a carrier peptide (6H-D3E) in the presense of 1 Mm IPTG. The 6H-D3E protein, approximately 55 kDa in size, was expressed in insoluble fraction which could be solubilized in lysis buffer containing strong denaturant, 6M guanidine.

Purification of the 6H-D3E protein could be obtained by immobolized metal affinity chromatography (IMAC) under denaturing condition. The purified 6H-D3E was eluted by acidic buffer containing 8M urea (Ph 4.5). The 6H-D3E refolding was carried out by dialyzing the denatured protein solution in the buffer containing stepwise dilution of urea with the addition of 0.1% Triton X-100 in stead of urea to the last buffer. The purified 6H-D3E was reactive to pooled sera of dengue patients (PCS) and 4G2 monoclonal antibody specific to flavivirus but not 10C10 monoclonal antibody specific to dengue 3 envelope protein. The *E.coli* contaminated protein (approximately 90 kDa) was found to be copurified with the 6H-D3E by western blot analysis to pooled sera of dengue patients (PCS). To obtain the purer 6H-D3E, the purification of 6H-D3E was modified by 1) washing the 6H-D3E pellet after sonication with the buffer containing 0.5% Triton X-100 and 2) increasing the volume of 8M urea lysis buffer Ph 8.0 to wash the 6H-D3E-bound resin before protein elution. The purified 6H-D3E after refolding was reactive to PCS with no background to pooled normal human sera (PND) by dot enzyme immunoassay (DEIA). The recombinant 6H-D3E demonstrated its potential to be used as antigen in development of immunological diagnostic tests.