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KOBPORN BOONNAK: PRODUCTION AND CHARACTERIZATION OF ANTIBODY AGAINST STRUCTURAL PROTEIN OF *AEDES ALBOPICTUS* DENSOVIRUS. THESIS ADVISORS: SA-NGA PATTANAKITSAKUL, D.Med.Sc., PRIDA MALASIT, MD, F.R.C.P.,110 P.ISBN 974-664-853-5.

Aedes albopictus densovirus (*Aal*DNV) is a small icosahedral, single-stranded DNA, non-enveloped virus which belongs to the family *Parvoviridae*. Two of the major densoviruses, the *Aedes aegypti* (*Aae*DNV) and *Aedes albopictus* densoviruses (*Aal*DNV) infected mosquitoes that were known to carry viruses responsible for the major public health diseases, namely dengue hemorrhagic fever and yellow fever.

This study involved the production of recombinant structural protein of *Aal*DNV from *E.coli* and the recombinant protein which was later used to raise specific antibody in rabbit. The gene segment of *Aal*DNV encoding for the structural protein was cloned into the bacterial plasmid, pMal-C2 containing a gene encoded for the 83 kDa maltose binding protein (MBP). The recombinant polyproteins containing both the *Aal*DNV structural protein and the MBP were produced in *E.coli*. The recombinant protein was purified by single-step affinity chromatography on an amylose resin. This purification protocol yielded about 0.16 mg of purified recombinant protein from 1 litre of shake flask culture. Antibody against structural protein of *Aal*DNV was produced by immunization the rabbit with purified recombinant protein. The rabbit antiserum strongly reacted against 41 kDa protein of *Aal*DNV-infected C6/36 cells as determined by western blot technique.

Use of this antibody in immunocytochemical staining of *Aal*DNV-infected cells revealed specific cytoplasmic staining patterns. Moreover, this antibody also stained general compartments of both mock and infected cells due to the high background brought by non-specific antibody to the mosquito cells. Absorption of rabbit antiserum with *Aedes albopictus* cells (C6/36) or *Toxorynchites splendens* mosquitoes did not decrease the non-specific staining to a satisfactory level. However, this antibody could not react with *Aedes aegypti* densovirus (*Aae*DNV), the closely related densovirus in the same group. The antibody has thus been used to identify the virus in infected cells solely by western blot technique.