Abstract

Dengue virus, a member of arbovirus that is transmitted by mosquito, is prevalent in tropic and subtropic area. The infection of dengue virus increases due to the effect of global warming, therefore, the development of early diagnostic reagents is very important to the prevention and treatment of dengue viral disease. In developed diagnostic kits, the envelope protein (E/M) and non-structural protein NS1 of dengue virus have been used as the antigens to detect IgM and IgG antibodies of patients. Dr. J.H. Huang, the cooperator of this project, has established an ELISA assay to analyze the patient's serum for early diagnosis. The IgM and IgG antibodies against E/M ptoein of dengue virus can distinguish primary and secondary infections. The IgG antibody against NS1 not only distinguished primary and secondary infections but also differentiated the serotypes of dengue virus from primary infection. According to the analysis of western blot and MIA, the NS1, NS3, and NS5 are the most important proteins to cause antibody reactions to dengue, Japanese encephalitis, and westnile viruses. Valdes, K *et al*., found that anti-E, -NS3, and –NS5 antibodies were present in the serum of patients that were infected by dengue virus type 2 and 4 in the primary or secondary stage. This indicates that NS3 and NS5 can be potential tools for the development of kits to diagnose dengue fever and dengue hemorrhagic fever.

To obtain the intracellular antigens NS3 and NS5, we had used *E. coli* system to express and isolate these proteins. However, these bacteria synthesized protein are not modified by glycosylation, acetylation, and phosphorylation, which may affect the antigenicity of protein. To solve this problem, in this project we established the baculovirus-insect system to overexpress NS3 and NS5 proteins and purifed these proteins by HiTrap chelating column. As a result, $NS3^{328\text{-}615}$, $NS5^{261\text{-}656}$, and $NS5^{261\text{-}900}$ containing portion of NS3 and NS5 proteins were obtained. Although these proteins are in denatured form, these exposed amino acid sequences still can be recognized by patient's antibodies. Therefore, these purified proteins are useful for the establishment of the diagnostic kits for dengue viral infection. In fact, through dialysis buffer, these denatured proteins also can be renatured to native form for diagnosis usage in future. Although obtaining the full-length NS3 and NS5 protein is one of our specific aims, the characteristics of rearrangement and deletion of these gene sequences in the expression vectors frustrated our plan. However, an interesting finding that the fusion of 40 bp at the upstream of these genes would not cause the instability of cloning vector suggested that the fusion of extra amino acid sequences at the upstream of NS3 and NS5 proteins might be the way to get the expression of full-length NS3 and

NS5 proteins.

Keywords: Dengue virus, NS3, NS5, baculovirus, insect cell.