CHAPTER VII

SUMMARY

Dengue virus is a member in the genus *Flavivirus*, the family *Flaviviridae*. A dengue virion contains a single-stranded positive RNA genome about 11 kb containing one long open reading frame which serves as a template for 3 structural proteins (C, prM and E) and 7 nonstructural proteins. The virion assembles at the ER membrane and an immature virion is then released into the ER lumen and transported through the secretory pathway. Shortly before the particle release, prM protein of the immature particle is cleaved at furin consensus sequence by furin, leaving the M portion on the particle and allowing maturation of the virion to occur.

The furin consensus sequence at pr-M junction is conserved in the flaviviruses, however the efficiency of cleavage of dengue virus prM has been shown to be lower than the others. A study by Keelapang et al., (2004) found that a chimeric virus, JEVpr/16681, carrying a replacement of the 13-amino acid sequence just proximal to the pr-M junction of Japanese encephalitis virus (JEV) in a dengue serotype 2 virus, strain 16681 showed an enhanced level of prM cleavage in an accompanying with a delayed export of infectious virus particles out of the infected cells. A comparison of amino acid residues in the replaced region of the JEVpr/16681 virus and the parental strain reveals three types of amino acid substitutions: 1) the substitution of uncharged residues at positions P8, P10 and P13 with arginine, 2) the substitution of glutamic acid at positions P3 and P7 with uncharged residues, and 3) the substitution of two uncharged residues at positions P6 and P9 with other uncharged residues. This leads to a question that which of these changes is(are) responsible for the enhanced prM cleavage and delay particle export found in the JEVpr/16681 virus. Therefore, three mutant viruses; 16681pr(+7,-2) harboring glycine-to-arginine, threonine-to-arginine and threonine(1)-to-arginine substitutions at P8, P10 and P13 positions; 16681pr(+4,-0)HS harboring glutamic acid-to-serine and glutamic acid-to-histidine substitutions at P3 and P7; and 16681pr(+7,-0) harboring

glutamic acid-to-histidine, glutamic acid-to-serine, glycine-to-arginine, threonine-to-arginine and threonine-to-arginine substitution at P3, P7, P8, P10 and P13, previously constructed by Songjaeng (2004) and a single point mutant virus, 16681prE203A constructed by Sittisombut (personal communication) were examined by protein labeling and a single-step kinetics assay.

Determination of the prM protein cleavage levels indicated that the absences of the negatively charged amino acid residues at the position P3 and P7 [in 16681pr(+4,-0)HS and 16681pr(+7,-0) viruses] enhanced the cleavage levels. The levels of cleavage of these mutants were equal to that of the JEVpr/16681 virus. In contrast, the cleavage level of 16681pr(+7,-2) virus had no different from the levels of the wild type virus strain 16681Nde(+).

Results of the particle export by the single-step kinetics assay showed that the extracellular virus titers of 16681pr(+7,-2) and JEVpr/16681 did decrease, even though the decreased level of 16681pr(+7,-2) was lesser than that of the JEVpr/16681 On the other hand, the virus titers of both the cell-associated and extracellular virus of 16681pr(+4,-0)HS and 16681prE203A viruses were not different from the wild type virus. These results indicated that the arginine residues at the P8, P10 and P13 reduced JEVpr/16681 particle export whereas the absence of the P3 and/or P7 negatively charged amino acid residues affected on the prM protein cleavage levels rather than the particle export.

To further clarify the positions of the arginine residues that might play an important role on JEVpr/16681 and 16681pr(+7,-2) viruses delaying the export. Three double mutant viruses of the arginine residues at the positions P8, P10 and P13 were generated. These mutant viruses were pr(+6,-2)P8,10+ harboring glycine-to-arginine and threonine-to-arginine amino acid substitutions at P8 and P10; pr(+6,-2)P10,13+ harboring threonine-to-arginine and threonine-to-arginine amino acid substitutions at P10 and P13; and pr(+6,-2)P8,13+ harboring glycine-to-arginine and threonine-to-arginine amino acid substitutions at P8 and P13. These mutant viruses were investigated in parallel with 16681Nde(+) and JEVpr/16681 by the single-step kinetics studies.

The results showed that the extracellular virus titers of 16681pr(+6,-2)P8,10+ or 16681pr(+6,-2)P10, 13+ were lower than those of the 16681Nde(+) and

16681pr(+6,-2)P8, 13+ viruses over 20-48 hours after infection, while their cell-associated virus titers revealed similar levels to those of the wild type virus. The reductions of the extracellular virus titers of 16681pr(+6,-2)P8,10+ or 16681pr(+6,-2)P10,13+ were not as much as those of 16681pr(+7,-2) and JEVpr/16681 did. This result indicated that the arginine residues at either P8 and P10 or P10 and P13 sufficiently delayed the particle export. But the presence of all the arginine residues at P8, P10 and P13 were stronger affected than those two sites.

In conclusion, it was demonstrated that the positively charged amino acid residues, the arginine residues, at P8, P10 and P13 caused reducing the particle export. Combination of those three arginine residues predominated than combination of the other two arginine residues. However, the P10 arginine that found in both 16681pr(+6,-2)P8,10+ and 16681pr(+6,-2)P10,13+ viruses maybe affected particle export could not be excluded. On other hand, the absences of P3 and P7 negatively charged amino acid residues that found in both 16681pr(+4,-0)HS and 16681prE203A viruses had a little effect on exporting phenotype although the cleavage experiment showed high level of prM cleavage as found in JEVpr/16681 virus.