I. INTRODUCTION

Dengue viruses are mosquito-borne human pathogens that represent a serious public health threat. The viruses are transmitted by *Stegomyia* mosquitoes, such as *Aedes aegypti* or *A. albopictus*. Diseases caused by dengue viruses range from a mild form of dengue fever to a more severe dengue hemorrhagic fever/shock syndrome, which causes considerable morbidity and mortality, especially among children in the tropical and subtropical regions of the world (Monath, 1994; Rigau-Pe'rez *et al.*, 1998).

Dengue viruses type 1-4 are members of the flavivirus family of positive-stranded RNA viruses. All of the four serotypes of dengue virus can cause classical dengue fever and dengue hemorrhagic fever (Henchal and Putnak, 1990) and there is no long-term cross-protective immunity between the dengue serotypes. Therefore, people may have as many as four dengue infections during their life. The genome of dengue virus and other flaviviruses consist of a single molecule of positive-stranded RNA, which is 10.5-11 kb in length. The viral genome encodes three structural and seven non-structural proteins, which are cleaved from a polyprotein precursor by host- and virus-encoded proteases (Rice, 1996).

The three viral proteins that contain *N*-linked glycans are located consecutively in the polyprotein. These are prM (precursor to the membrane protein M), E (the envelope protein) and the non-structural protein NS1. The proteolytic cleavages, which produce the *N*-termini of these proteins, are mediated by the host signalase enzyme. The *C*-termini of prM and E are also generated by signalase cleavages. Cleavage at the *C*-terminus of NS1 occurs at a site that meets the requirements for signalase cleavage, but lacks an upstream membrane anchor (Wright *et al.*, 1989). It appears that this cleavage is effected by a host protease, which may actually be signalase, in the endoplasmic reticulum (Falgout and Markoff, 1995).

The prM glycoprotein is cleaved to produce the mature M protein just before or at the time of virion release from cell (Murray *et al.*, 1993). This internal cleavage of prM is effected by the host furin proteases (Stadler *et al.*, 1997). The prM and E proteins form heterodimers in cell-associated virions (Wengler and Wengler, 1989). These prevent premature acid-induced conformational changes of the E protein during virion maturation in the *trans*-Golgi network and secretory vesicles (Heinz *et al.*, 1994). The immature virions, of which the proteolytic cleavage of prM to M in the late stage of the replication cycle were inhibited by treatment of infected cells with acidotropic amines, are less infectious than mature ones (Randolph *et al.*, 1990). And then, these immature virions are unable to fuse with C6/36 mosquito cells in a low pH environment (Guirakhoo *et al.*, 1992).

For the dengue viruses, the genomic length cDNA clones have been constructed, which can be transcribed yield infectious RNA. This has greatly facilitated the study of dengue replication and protein function in infected cells. The genomic length of dengue virus cDNA, yielding infectious RNA, is available for the Western Pacific 74 strain of dengue serotype 1 (Puri *et al.*, 2000), the New Guinea C (NGC) strains of dengue serotype 2 (Kapoor *et al.*, 1995; Polo *et al.*, 1997; Gualano *et al.*, 1998), the 16681 strain of dengue serotype 2 (Kinney *et al.*, 1997; Sriburi *et al.*, 2001), and the 814669 strain of dengue serotype 4 (Lai *et al.*, 1991).

In a previous study, Sittisombut and Sriburi (unpublished data) constructed full-length cDNA clones of eight glycine-scanning dengue mutants in which each of the amino acid residues 199 to 206 around the prM-M junction of dengue serotype 2 virus was mutated to glycine. The genomic-length capped RNA, which were transcribed from these eight full-length cDNA mutant clones, were transfected into C6/36 mosquito cells. They found that three glycine-scanning mutants of the basic amino acid positions 202, 204 and 205, were not viable. On the other hand, additional

mutations of the positions 199, 200, 201, 203 and 206 did not affect dengue virus replication.

To further determine the requirement for basic amino acid at the amino acid residues 204 and 205 of dengue serotype 2 strain 16681 (in the prM-M cleavage site) by proprotein convertase during the multiplication of dengue virus in the C6/36 mosquito cell line, the two basic residues just proximal to the prM-M cleavage site [the amino acid residue 204 (lysine) and 205 (arginine)] of dengue serotype 2 were mutated by site-directed mutagenesis into five different amino acids containing either a weakly basic side chain (histidine), an alternative strongly basic side chain (arginine for position 204 and lysine for position 205), an acidic side chain (aspartic acid), an uncharged polar side chain (serine), or a non-polar side chain (alanine). The strategy of Sriburi *et al.* (2001) was used in this study to construct these mutation-containing full-length cDNA clones and to initiate virus replication in C6/36 cells by transfection. Production of the viral proteins and infectious virus was then monitored in supernatant of transfected mosquito cell line for up to 8 weeks to explore the effect of these mutations on virus replication.