Original article

Effect of acidic amino acid substitution at the pr-M junction of dengue type 2 virus on prM cleavage and virus replication

Rungtawan Sriburi, Tassanee Rattanapak, and Nopporn Sittisombut

Department of Microbiology, Faculty of Medicine, Chiang Mai University

Cleavage by furin of the envelope glycoprotein, prM, is required for maturation and infectivity of the dengue virus. Highly conserved acidic amino acid, aspartate in DENV-1 and -3 or glutamate in DENV-2 and -4, at a P3 position proximal to the furin cleavage site, influences the level of prM cleavage during virus replication. However, the significance of this inter-serotypic variation of P3 acidic residue has not been elucidated. The aim of this study was to define the effect of acidic amino acid variation at this P3 position in the pr-M junction on the extent of prM cleavage and virus replication. The mutant virus, strain E203D, containing a P3 glutamate-to-aspartate substitution was generated by site-directed mutagenesis of a full-length cDNA clone of the dengue serotype 2 virus, strain 16681. The level of prM cleavage, as determined by the metabolic labeling method employing ^{35}S-Cys/Met, in the strain E203D was comparable to the parent virus. When virus replicative ability was assessed in single and multi-step multiplication studies, no difference existed in the kinetics of replication between the strain E203D and the parent virus. A glutamate-to-aspartate substitution in prM at the polyprotein position 203 does not appear to affect the level of prM cleavage or viral replication in the dengue serotype 2 virus. **Chiang Mai Medical Journal 2016;55(Suppl 1):1-9.**

keywords: dengue, prM cleavage, site-directed mutagenesis, virus replication

Introduction

Dengue virus is an enveloped RNA virus belonging to the genus *Flavivirus* of the family *Flaviviridae*[1]. Four serotypes of dengue virus (DENV-1, -2, -3 and -4) are classified based on their antigenic characteristics[2] and sequence homology. Dengue virion is composed of three structural proteins; capsid (C), membrane precursor/membrane (prM/M) and envelope (E). The two envelope proteins, prM/M and E, are embedded in the lipid bilayer. Viral nucleocapsid contains the C protein and a positive-sense single-stranded RNA genome of approximately 10.7 kb[3]. During dengue virus replication in host cells, immature particles are exported through the secretory pathway. Prior to release, prM is cleaved by the cellular enzyme, furin, which leads to conversion of immature particles into mature ones[4].

Cleavage of prM is catalyzed by furin, which recognizes the consensus sequence,

Address correspondence to: Nopporn Sittisombut, M.D., Ph.D., Department of Microbiology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200 Thailand. E-mail: nopporn.sittiso@cmu.ac.th

Received: July 22, 2015**; Accepted:** August 24, 2015

Arginine(P4)-X(P3)-Lysine/Arginine(P2)-Arginine(P1), where X can be any amino acid[5]. Although, all prM molecules on the surface of immature particles contain this consensus sequence, only some of them are cleaved in extracellular particles[6]; such partial cleavage is due partially to the amino acid sequence at the non-consensus positions. A negatively charged amino acid at the P3 position has adverse effects on the level of prM cleavage[7]. Acidic amino acid at the P3 position of the furin cleavage site in the prM of dengue virus is highly conserved, but occurs as aspartate in DENV-1 and -3 or glutamate in DENV-2 and -4[6]. The significance of alternating the two acidic amino acids at the P3 position of the prM cleavage site among various dengue serotypes is unknown. This study examined the influence of this amino acid variation by constructing the E203D mutant of DENV-2 and determining the effect of this mutation substitution on the extent of prM cleavage and viral replication.

Materials and methods

Cell lines

C6/36 cells, a mosquito cell line derived from *Aedes albopictus*[8], were grown at 29 °C in Leibovitz's L-15 medium containing 10% heat inactivated fetal bovine serum (FBS), 1x Glutamine (Gibco, Life Technologies, Carlsbad, CA, USA), 1x penicillin/streptomycin solution (Gibco) and 0.29% tryptose phosphate broth. Vero cells, an African green monkey kidney cell line (ATCC CCL-81), were grown in minimal essential medium (MEM) containing 10% FBS, 1x Glutamine, 1x penicillin/streptomycin solution and 0.22% sodium bicarbonate. Vero cells were incubated at 37 ºC in the presence of 5% CO_2.

Viruses

The dengue virus serotype 2 strain 16681 was isolated in Thailand during 1964 from a patient with dengue hemorrhagic fever[9]. This strain was kindly provided by Drs. Bruce Innis and Ananda Nisalak from the Department of Virology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand. A recombinant derivative of strain 16681, 16681*Pst*(-), was generated from a full-length cDNA plasmid clone of strain 16681 by employing site-directed mutagenesis to abolish a *PstI* site at the nucleotide position 402[6,10] without affecting the amino acid sequence.

Antibodies

3H5, an IgG1 specific for DENV-2 E protein[11], and 4G2, an IgG2a isotype specific for flavivirus E protein[11] were used in focus immunoassay titration and indirect immunofluorescence analysis. An alkaline phosphatase-conjugated goat IgG, specific for mouse IgG (H+L) (Zymed, San Francisco, CA, USA), was used as a secondary antibody in focus immunoassay titration. Cy3-conjugated goat anti-mouse IgG antibody (Invitrogen, Camarillo, CA, USA) was used in indirect immunofluorescence.

Construction of the E203D mutant virus

The E203D mutation was introduced into the pr-M junction of a full-length cDNA clone of the 16681 strain[10] by the polymerase chain reaction (PCR)-based site-directed mutagenesis method (QuikChange, Stratagene), using specific primers (Table 1) and a 5' half genome (pBK(S1SP6-1547)Δ402*Pst*)[10] as a template. The 5' half genome plasmid, containing the mutated sequence, was linearized by *KpnI* digestion, and then the full-length cDNA clone was generated by ligating a *KpnI* fragment of the 3' half genome[10] to the *KpnI*-digested 5' half genome. The ligated products were

Table 1. Primers for site-directed mutagenesis, RT-PCR and nucleotide sequence analysis

Reaction	Primer	Sequence (5'→3')	Nucleotide position[a]	Reference
Site- directed mutagenesis	E203D-F	GAAGAGACAAAAGATCTGTGGCACTCGTTCCACAT	698-732	This paper
	E203D-R	GCCACAGATCTTTTGTCTCTTCTATGTTCTCCCATGG	683-719	
RT-PCT	D2J134	TCAATATGCTGAAACGCGAGAGAAACCG	134-162	[16]
	D2J2504	GGGGATTCTGGTTGGAACTTATATTGTTCTGTCC	2504-2471	
Sequencing analysis	S350	GGAAAGAGATTGGAAGGATGA	350-371	[10]

[a], position of nucleotide in the DENV-2 genome is based on Blok et al[17].

transformed into the *E. coli* strain DH5αF' (Invitrogen), and amplified in the presence of 25 μg/mL Ampicillin at 25 °C[10]. Synthesis of capped *in vitro* RNA transcripts and generation of the mutant virus from RNA transcripts in C6/36 cells were performed as described previously[10].

Virus propagation

Viruses obtained from day 7 after the transfection were propagated in C6/36 cells in a 25-cm^2 tissue culture flask. Culture supernatants collected at day 5 and 7 after infection were stored in 20% FBS at -70 °C in a small aliquots for single use. Infectious viral titer was quantified by focus immunoassay titration.

Indirect immunofluorescence

Transfected cells on the coverslips were washed with phosphate-buffered saline (PBS) and fixed with 3.7% formaldehyde in PBS, followed by permeabilization with 2% Triton X-100[6]. Intracellular E proteins were detected by using 4G2, followed by Cy3-conjugated goat anti-mouse IgG antibody and 4'-6-diamidino-2-phenylindole (DAPI), and visualized under a fluorescence microscope (Provis AX, Olympus, Melville, NY, USA).

Focus immunoassay titration

Culture supernatant was diluted serially in ten-fold steps with MEM containing 2% FBS, and 50 μL of each virus dilution were added to duplicate wells of the confluent Vero cells in a 96-well plate. Adsorption was carried out at 37 °C for 2 hr with intermittent manual shaking. The cell monolayer was overlaid with an overlay medium containing MEM supplemented with 2% FBS, 1.2% carboxymethyl cellulose and 1x penicillin/streptomycin solution, and the plate was incubated at 37 °C. After three days of incubation, the cells were fixed with 3.7% formaldehyde in PBS and permeabilized with 2% Triton X-100. Clusters of infected cells were detected by using 3H5, alkaline phosphatase-conjugated goat anti-mouse IgG antibody and nitro blue tetrazolium chloride/5-bromo-4-chloro-3-indolyl phosphate mixture, successively. Infectious virus titer was expressed in focus forming unit (FFU) per milliliter.

Nucleotide sequence analysis

The viral genomic RNA was extracted from culture supernatant of the third C6/36 passage by using the viral nucleic acid extraction kit (Geneaid Biotec Ltd., New Taipei City, Taiwan). The prM coding region was amplified by reverse transcription-PCR reaction using specific primers (Table 1). Nucleotide sequence analysis was performed by using the Sanger dideoxy chain terminator method.

Metabolic labeling of viruses and quantitation of prM cleavage

The confluent monolayer of C6/36 cells was infected at a multiplicity of infection (MOI) of 0.2. The culture medium was replaced at 48 hr after infection with methionine- and cysteine-free-Dulbecco's modified Eagle medium containing 1.5% dialyzed FBS, and incubated for 1 hr at 37 °C before adding 100 μCi of L-[^{35}S] methionine and L-[^{35}S] cysteine (PerkinElmer, Waltham, MA, USA). At 24 and 48 hr after labeling, the culture supernatant was concentrated and centrifuged through a 5–25% sucrose gradient[7]. Three adjacent sucrose fractions with high radioactivity were pooled, concentrated and separated by electrophoresis in a 0.1% SDS-15% polyacrylamide gel, which was exposed to a phosphorimager screen (Amersham, Pittsburgh, PA, USA) after drying. The radioactivity of labeled viral protein bands was measured by using a phosphorimager (Typhoon 6410, Amersham) and analyzed with Image Quant software. Radioactivity signals of prM and M bands were subtracted from local background signals and then adjusted by dividing the radioactivity with the numbers of cysteine and methionine in each protein, in order to eliminate differences in the number of methionine and cysteine residues present in prM and M[6]. Adjusted radioactivity signals of prM and M were then used in the calculation of prM cleavage with the following formula:

prM cleavage (%)=[M adjusted signal/(prM adjusted signal + M adjusted signal)] × 100.

Virus multiplication study

In a single-step multiplication study, subconfluent Vero cells were infected with dengue virus in a 35-mm^2 culture dish at an MOI of 1, and washed five times with 1x MEM 2 hr after adsorption, before a maintenance medium containing 2% FBS, 1x Glutamine and 1x penicillin/streptomycin solution was added to the monolayer. Culture supernatant was collected at different time points for 48 hr after infection and stored at -70 °C. Infectious viruses were quantitated using focus immunoassay titration. Similar steps were performed in a multistep study, except for the use of an MOI of 0.2 for both Vero and C6/36 cells. Infectious virus titer was measured daily for seven days in the multistep study.

Statistical analysis

The two-tailed student's *t* test was used to compare the extent of prM cleavage and virus replication kinetics. *p* values of less than 0.05 were considered as significant.

Results

Generation and characterization of the E203D mutant virus

The E203D mutation was introduced into the prM coding region of the full-length cDNA plasmid clone of the strain 16681[10] using PCR site-directed mutagenesis. Following an *in vitro* transcription reaction, transcription products were transfected into C6/36 cells and the presence of a mutant virus in the transfected cells was monitored by indirect immunofluorescence analysis. Cell fusion was observed at day 9 after transfection, and E protein of the dengue virus was detected in the cytoplasm of transfected, but not mock transfected, C6/36 cells (Fig.1A). After three amplification passages in the C6/36 cells, infectious titer

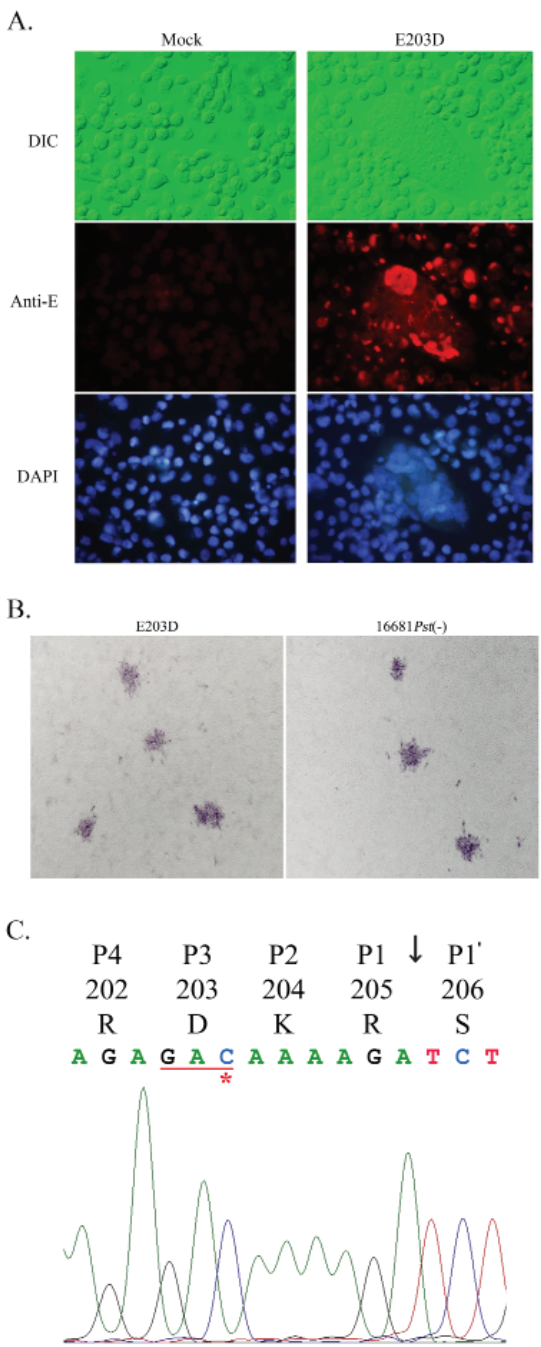

Figure 1. Characterization of the E203D mutant virus. (A) Detection of dengue viral E protein in the transfected C6/36 cells by indirect immunofluorescence staining. C6/36 cells were transfected with *in vitro* RNA transcripts generated from a full-length cDNA clone with the E203D mutation. Cells at day 9 after transfection were stained with the anti-E antibody, 4G2, followed by Cy3-conjugated goat anti-mouse IgG antibody. DIC, differential interference contrast. (B) Representative foci of E203D and 16681*Pst*(-), as observed in infected Vero cells in focus immunoassay titration. (C) Nucleotide and amino acid sequences of the pr-M junction of the E203D strain, which was subjected to nucleotide sequence analysis from the third C6/36 passage. The underlined (GAC) indicates the nucleotide sequence at positions 703 to 705 of the viral genome. (*) shows the substituted A→C base at the nucleotide position 705. Numbers 202-206 represent amino acid positions of the polyprotein. P1' and P1-P4 indicate the furin cleavage positions. (↓) furin cleavage site. The single letter nomenclature of amino acid was employed.

Table 2. Properties of virus strains employed in this study

Strain	Designation	Titer (FFU/mL)	% prM cleavage[a] [mean (SD)]
16681	16681	$8.72x10^{7}$[b]	45.5 (3.9)
16681pr(E203D)	E203D	1.62×10^{7}[c]	36.3 (6.1)

a, n=2, p >0.05; b, harvested on day 4 of the second passage in C6/36 cells at an MOI of about 0.01; c, harvested on day 7 of the third passage in C6/36 cells at an MOI of about 0.05.

of the E203D strain was in the same order of magnitude as the parent virus strain 16681 (Table 2). Similar focus size was observed between the E203D strain and parent virus in focus immunoassay titration (Fig. 1B). Nucleotide sequence analysis revealed an introduced A→C substitution at position 705 of the E203D strain genome (Fig. 1C). This substitution was intended to result in a glutamate-to-aspartate replacement at the polyprotein position 203 just proximal to the pr-M cleavage site. No other mutation was found in the remaining prM sequence.

Comparison of prM cleavage

In the assessment of prM cleavage, extracellular progeny virus particles were labeled metabolically, and viral particles were partially purified by rate-zonal centrifugation. Viral proteins were disrupted and separated by SDS-PAGE (Fig. 2), and the proportion of prM and M in the viral particles was determined by using a phosphoimager. As shown in Table 2 and 3, comparisons of adjusted radioactivity signals of prM and M bands revealed that the extent of prM cleavage of the strain E203D was not different from that of the parent virus (p> 0.05), indicating that the glutamate-to-aspartate substitution at the polyprotein position 203 did not affect the level of prM cleavage during the multiplication of DENV-2 in C6/36 cells.

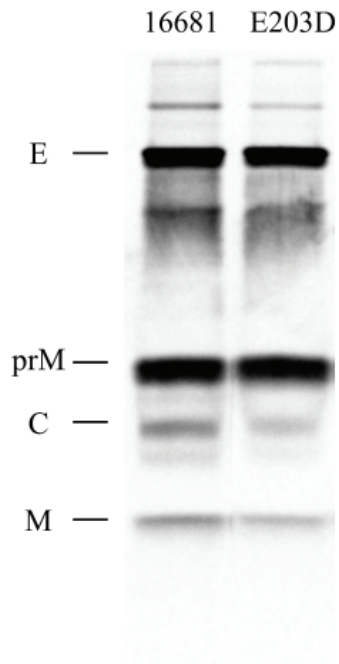

Figure 2. Comparison of prM cleavage between the strains 16681 and E203D. Virus-infected C6/36 cells were labeled with L-[^{35}S]methionine and L-[^{35}S] cysteine. Extracellular virus particles were partially purified by rate-zonal centrifugation, lysed and then separated by SDS-PAGE. Dried gel was exposed to phosphor screen. Viral proteins (E, prM, C and M) are indicated. E, prM, M and C proteins were identified based on their electrophoretic mobilities and sizes.

Effect of E203D mutation on virus replication

Replication of the E203D strain was compared with the parent DENV-2 virus[10] in Vero cells by employing single step and multistep kinetic analyses (Fig. 3). In the single step kinetic study, cells were infected at a high MOI and infectious viruses were measured in the culture supernatant at various time points for 48 hr (Fig. 3A). Similarly, the elevated level of E203D and parent viruses was first detected at 12 hr after infection, with the plateau levels reached at 24 hr (Fig. 3A). In the multistep

Table 3. Assessment of prM cleavage

Experiment	Virus	Protein	Total radioactivity signal[a]	Adjusted radio-activity signal[b]	Ratio of M and (prM+M) adjusted radioactivity signal	Level of prM cleavage (%)
1	16681	prM	18,737,825	1,171,114	0.4832	48.3
		M	5,474,860	1,094,972		
	E203D	prM	44,219,020	2,763,689	0.4055	40.6
		M	9,425,684	1,885,137		
2	16681	prM	53,081,893	3,317,618	0.4267	42.7
		M	12,346,894	2,469,379		
	E203D	prM	54,792,820	3,424,551	0.3185	31.9
		M	8,004,088	1,600,818		

[a], after background subtraction; [b], after dividing with the numbers of methionine and cysteine in prM or M.

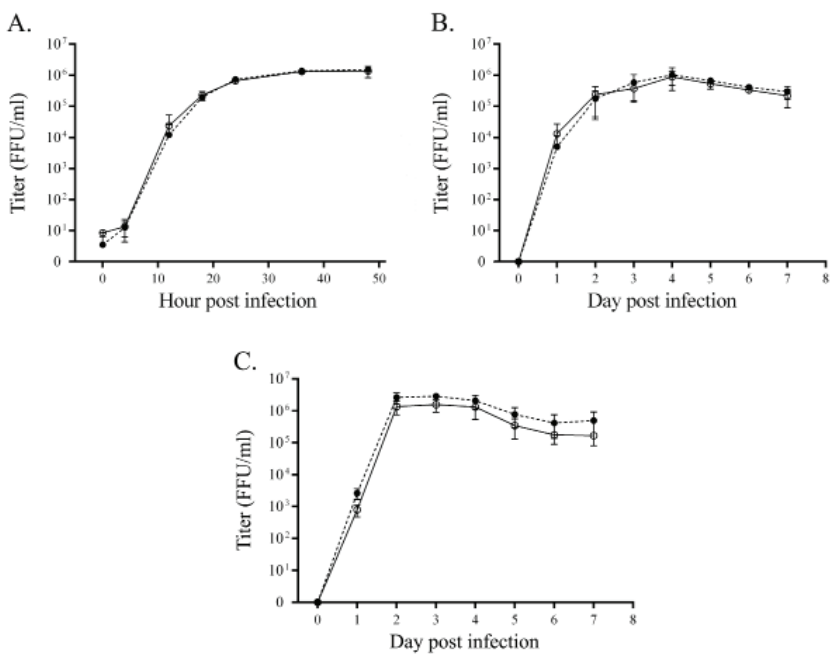

Figure 3. Comparison of replication between the strains 16681 and E203D. Vero (A, B) or C6/36 cells (C) were infected at an MOI of 1 (A) or 0.2 (B, C), washed extensively and the culture supernatants were collected at indicated time points. Infectious virus titer was determined at each time point by focus immunoassay titration in Vero cells. Data represent the mean with SEM from two or three separate experiments. Closed circle (●) = 16681*Pst*(-), Open circle (○) = E203D.

kinetic study (Fig. 3B), titers of both strains increased rapidly during the first two days of infection and remained at high levels thereafter. Similar results were observed when the multistep kinetic study was performed in C6/36 cells (Fig. 3C). These results indicated that the strain E203D was similar to the parent virus in its replicative ability, when assessed in Vero and C6/36 cells.

Discussion

The influence of acidic amino acid variation at the P3 position of a furin cleavage site on

the levels of prM cleavage and viral replication was investigated in this study by introducing a glutamate-to-aspartate substitution at the position 203 of the DENV-2 virus polyprotein. The level of prM cleavage, focus size and replication kinetics of the mutant was similar to that of the parent virus, thus amino acid at the 203 position of the prM protein of DENV-2 could be either glutamate or aspartate. This result was not surprising because glutamate and aspartate can substitute for each other in many proteins; and this represents an example of conservative amino acid substitution[12,13]. Similar conservative substitution occurs in the cleavage site of an enteropeptidase enzyme, which cleaves trypsinogen into trypsin[14,15].

While similar levels of prM cleavage were observed between the strains E203D and 16681, the level of prM cleavage in the 16681 strain was lower than those that we reported previously[6,7]. Changes in extent of prM cleavage in the 16681 strain are likely due to technical variability in this study such as unequal labeling of viral proteins with radioactive precursors procured from different suppliers, or different proportions of methionine and cysteine in various batches of isotope used. An additional source of variation may arise while determining prM cleavage. The radioactivity signal of the protein band is calculated from a selected area after a phosphorimager screen has been scanned, and if the protein band does not migrate as a sharp band, the areas selected in each experiment could affect the calculation.

Cleavage of the prM protein is involved in acquiring infectivity of dengue virus particles. Mutations that were introduced at the pr-M junction to alter the extent of prM cleavage may also influence other aspects of dengue virus replication. Our previous study of the pr-M junction chimeric virus, JEVpr/16681, revealed that an enhanced prM cleavage level was associated with a reduced level of infectious extracellular particles as well as delayed virus replication[6]. In a study of pr-M junction mutants[7], an enhanced level of the prM cleavage was observed in the mutant virus E203A although its replication was very similar to that of the parent virus. Unlike previous studies, the glutamate-to-aspartate substitution in this study had no impact on either the prM cleavage or replication. The levels of pr-M cleavage vary between all four serotypes of dengue virus, but there is no correlation with the P3 amino acid sequence and the extent of cleavage (unpublished data). The effect of glutamate-to-aspartate substitution in this study is limited to the DENV-2 background, and it would be of interest to determine whether this substitution would be different if employed on non DENV-2 backgrounds. Taken together with a strong conservation at the furin P3 consensus position, the results of this study are compatible with the founder effect that occurred during divergent evolution of separate ancestors, which gave rise to DENV-1 and -3, or DENV-2 and -4.

Acknowledgements

We thank Dr. Poonsook Keelapang, Dr. Utaiwan Utaipat and Amporn Suphatrakul for their helpful suggestions, and Tongkham Taya for his technical assistance. This investigation was supported by the National Science and Technology Development Agency, Thailand (BT-B-01-MG-10-5018). There is no conflict of interest.

References

1. **Pierson TC, Diamond MS**. *Flavivirus*es. In: Knipe DM, Howley DE, editors. Fields virology. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2013. p. 747-94.
2. **Calisher CH, Karabatsos N, Dalrymple JM, et al**. Antigenic relationships between *Flavivirus*es as determined by cross-neutralization tests with polyclonal antisera. J Gen Virol 1989;70(Pt1):37-43.
3. **Kuhn RJ, Zhang W, Rossmann MG, et al.** Structure of dengue virus: implications for *Flavivirus* organization, maturation, and fusion. Cell 2002; 108(5):717-25.
4. **Stadler K, Allison SL, Schalich J, Heinz FX**. Proteolytic activation of tick-borne encephalitis virus by furin. J Virol 1997;71(11):8475-81.
5. **Hosaka M, Nagahama M, Kim WS, et al**. Arg-X-Lys/Arg-Arg motif as a signal for precursor cleavage

catalyzed by furin within the constitutive secretory pathway. J Biol Chem 1991;266(19):12127-30.
6. **Keelapang P, Sriburi R, Supasa S, et al.** Alterations of pr-M cleavage and virus export in pr-M junction chimeric dengue viruses. J Virol 2004;78 (5):2367-81.
7. **Junjhon J, Lausumpao M, Supasa S, et al**. Differential modulation of prM cleavage, extracellular particle distribution, and virus infectivity by conserved residues at nonfurin consensus positions of the dengue virus pr-M junction. J Virol 2008; 82(21):10776-91.
8. **Igarashi A**. Isolation of a Singh's Aedes albopictus cell clone sensitive to Dengue and Chikungunya viruses. J Gen Virol 1978;40(3):531-44.
9. **Halstead SB, Simasthien F**. Observations related to the pathogenesis of dengue hemorrhagic fever. II. Antigenic and biologic properties of dengue viruses and their association with disease response in the host. Yale J Biol Med 1970;42(5):276-92.
10. **Sriburi R, Keelapang P, Duangchinda T, et al.** Construction of infectious dengue 2 virus cDNA clones using high copy number plasmid. J Virol Methods 2001;92(1):71-82.
11. **Henchal EA, Gentry MK, McCown JM, Brandt WE**. Dengue virus-specific and *Flavivirus* group determinants identified with monoclonal antibodies by indirect immunofluorescence. Am J Trop Med Hyg 1982;31(4):830-6.
12. **Betts MJ, Russell RB**. Amino acid properties and consequences of substitutions. Bioinformatics for geneticists. In: Barnes MR, Gray IC, editors. Bioinformatics for Geneticists. Chichester: John Wiley & Sons, Ltd; 2003. p. 289-316.
13. **French S, Robson B**. What is conservative substitution? J. Mol Evol 1983;19(2):171-5.
14. **Reeck GR, Neurath H**. Pancreatic trypsinogen from the African lungfish. Biochemistry 1972;11(4): 503-10.
15. **de Haën C, Walsh KA, Neurath H**. Isolation and amino-terminal sequence analysis of a new pancreatic trypsinogen of the African lungfish Protopterus aethiopicus. Biochemistry 1977;16(20): 4421-5.
16. **Lewis JA, Chang GJ, Lanciotti RS, Kinney RM, Mayer LW, Trent DW**. Phylogenetic relationships of dengue-2 viruses. Virology 1993; 197:216-24.
17. **Blok J, McWilliam SM, Butler HC**, **et al.** Comparison of a dengue-2 virus and its candidate vaccine derivative: sequence relationships with the *Flavivirus*es and other viruses. Virology 1992;187:573-90.

ผลของการแทนที่กรดอะมิโนที่มีคุณสมบัติเป็นกรดที่บริเวณรอยต่อของโปรตีน pr-M ของเชื้อไวรัสเด็งกี่ ไทป์ 2 ต่อระดับการตัดโปรตีน prM และการเพิ่มจำนวนของเชื้อ

รุ้งตะวัน ศรีบุรี, ทัศนีย์ รัตนภาค, และ นพพร สิทธิสมบัติ
ภาควิชาจุลชีววิทยา คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่

การตัดโปรตีน prM โดยเอ็นไซม์ฟิวรินมีความสำคัญต่อการประกอบเป็นอนุภาคไวรัสที่สมบูรณ์และความสามารถในการติดเชื้อของเชื้อไวรัสเด็งกี่ กรดอะมิโนที่มีคุณสมบัติเป็นกรดที่ตำแหน่ง P3 เหนือตำแหน่งการตัดของเอ็นไซม์ฟิวรินมีอิทธิพลต่อระดับการตัดโปรตีน prM ในระหว่างการเพิ่มจำนวนของเชื้อ โดยกรดอะมิโนที่มีคุณสมบัติเป็นกรดที่พบที่ตำแหน่ง P3 นี้มีความอนุรักษ์สูงโดยจะพบเป็นกรดแอสปาร์ติกในเชื้อไวรัสเด็งกี่ซีโรไทป์ 1 และ 3 หรือกรดกลูตามิคในเชื้อไวรัสเด็งกี่ซีโรไทป์ 2 และ 4 อย่างไรก็ตามยังไม่มีการศึกษาถึงความสำคัญของการมีกรดอะมิโนที่มีคุณสมบัติเป็นกรดที่แตกต่างกันในตำแหน่ง P3 นี้ในเชื้อไวรัสเด็งกี่ที่ต่างซีโรไทป์กัน งานวิจัยนี้จึงได้ทำการศึกษาบทบาทของกรดอะมิโนที่มีคุณสมบัติเป็นกรดที่ตำแหน่ง P3 บริเวณรอยต่อของโปรตีนย่อย pr และ M ของเชื้อไวรัสเด็งกี่ต่อระดับการตัดโปรตีน prM และการเพิ่มจำนวนของเชื้อ โดยสร้างเชื้อสายพันธุ์ E203D ที่มีการแทนที่กรดกลูตามิคที่ตำแหน่ง P3 ของโปรตีน prM ด้วยกรดแอสปาร์ติกด้วยวิธี site-directed mutagenesis โดยใช้ full-length cDNA clone ของเชื้อไวรัสเด็งกี่ซีโรไทป์ 2 สายพันธุ์ 16681 เป็นแม่แบบ เมื่อทำการศึกษาการตัดของโปรตีน prM ของเชื้อโดยการติดฉลากโปรตีนของเชื้อไวรัสด้วยสารกัมมันตรังสีและเปรียบเทียบการเพิ่มจำนวนของเชื้อโดยวิธี single step kinetic และ multi-step kinetic พบว่าระดับการตัดของโปรตีน prM และการเพิ่มจำนวนของเชื้อไวรัสเด็งกี่ซีโรไทป์ 2 สายพันธุ์ E203D ไม่แตกต่างจากเชื้อไวรัสต้นตอ ดังนั้นการแทนที่กรดกลูตามิคด้วยกรดแอสปาร์ติกที่ตำแหน่ง 203 ของโปรตีน prM ไม่มีผลต่อระดับการตัดของโปรตีน prM และการเพิ่มจำนวนของเชื้อไวรัสเด็งกี่ซีโรไทป์ 2
เชียงใหม่เวชสาร 2559;55(ฉบับเสริม 1):1-9.

คำสำคัญ: เชื้อไวรัสเด็งกี่ การตัดโปรตีน prM การทำให้เกิดการกลายพันธุ์เฉพาะที่ การเพิ่มจำนวนของเชื้อไวรัส