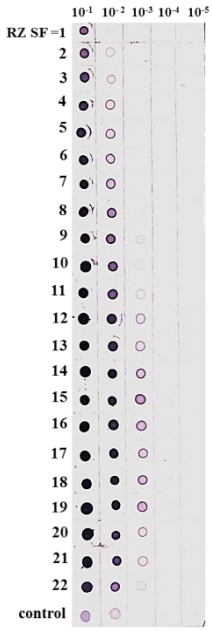

Fig. 3 Detection of extracellular E protein. The four fractions (2-5) from sucrose step gradient were separated by rate zonal centrifugation using a 5–25% (w/w) sucrose gradient. Fractions 1-22 were employed at several dilutions for the detection of extracellular E protein by anti-E (1D10) antibody and alkaline phosphatase-conjugated goat anti-mouse IgG antibody.

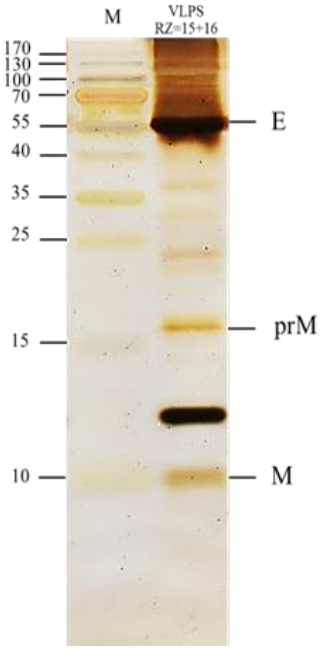

Fig. 4 SDS-PAGE analysis and silver staining. The rate zonal separation (fractions 15+16) were disrupted with 0.1% SDS, separated in a 5- 15% sodium docecylsulfate poly-acrylamide gel electrophoresis and stained with silver nitrate (lane 2). The viral structural proteins were indicated based on size. Sizes (in kD) of the protein marker bands (lane M) are indicated.

This result suggests that, in the presence of co-expressing prM, the DENV-4/2 EDIII chimeric E protein can assemble into particles that are released from transfected cells.

Characterization of VLPs

Several fractions derived from rate zonal centrifugation in sucrose density gradient were lysed using sodium dodecylsulfate and subjected to electrophoretic separation in sodium dodecylsulfate-polyacrylamide gel. Staining of the gel with silver nitrate revealed three major bands corresponding in size to the three viral proteins: E (54 kDa), prM (19 kDa) and M (8 kDa) (Fig. 4).

The simultaneous presence of these viral structural proteins in the same selected fractions form rate zonal centrifugation further indicated assembly of the chimeric E proteins into particulate form. The extent of prM cleavage among these particles as determined from the proportion of adjusted prM and M intensity signal was 71.8%, which was slightly

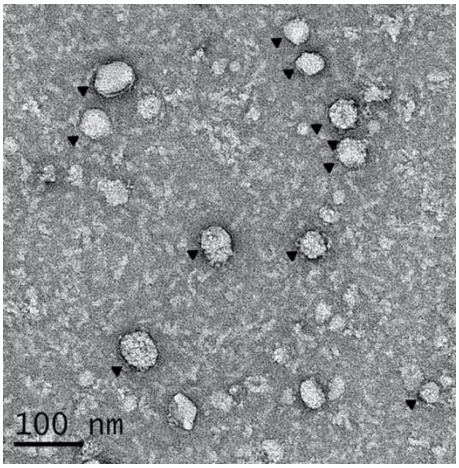

Fig. 5 Transmission electron microscopic visualization of chimeric VLPs particles. The rate zonal sucrose fractions 15+16 were fixed with 1% glutaraldehyde and stained with 1% uranyl acetate. Bar represents 100 nm. Black arrows indicate particles.

lower than the level of 83.2% observed in the parental DENV-2 VLPs (Suphatrakul et al., 2015). This result suggests that the substitution of DENV-4 EDIII domain in DENV-2 VLPs does not strongly affect the efficiency of prM cleavage. The result is consistent with a close similarity between DENV-2 and DENV-4 structural proteins as compared with other serotypes (Volk et al., 2007).

Analysis of the rate zonal sucrose fractions 15+16 by negative staining and transmission electron microscopy revealed spherical particles with wide variations (range 22-68 nm) in the diameter (Fig. 5). When the frequency distribution of 461 particles was plotted according to size, the distribution pattern was compatible with either a single size class of 34-48 nm particles, or two size subclasses of medium (34-39 nm) and large (41-48 nm) particles (Fig. 6).

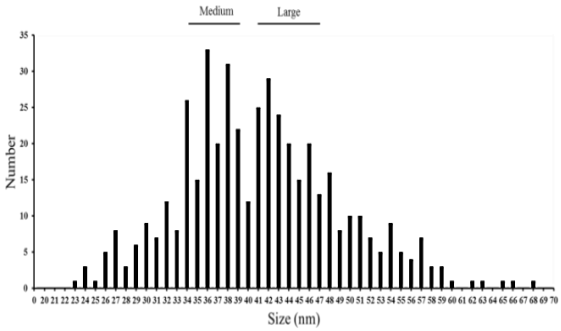

Fig. 6 The frequency distribution of particles. The particles (n= 461) were separated into 1 nm increment of the diameter. Bars indicate the peak frequency of the two possible size subclasses.

Large spherical particles detected in this preparation were similar in size to those previously described in the parental DENV-2 VLPs (Suphatrakul et al., 2015), DENV-4 VLPs (Zhang et al., 2011) and native viral particles of DENV-2 strain 16681Nde(+) (Junjhon et al., 2008). Whether the medium size particles represent a new class of VLPs, or derived from large VLPs by drying during the processing of the specimen for electron microscopy will need to be investigated further.

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