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Structural analysis of a “size switch type repacking” during the evolution of dengue envelope protein domain III (ED3)

Montasir Elahi , Mohammad M Islam, Yutaka Kuroda

Department of Biotechnology and Life Sciences, Tokyo University of Agriculture and Technology, Tokyo 184-8588, Japan.

1 Introduction

Dengue viruses (DEN) are classified into four serotypes (DEN1-DEN4) exhibiting high sequence and structural similarities, and infections by multiple serotypes can lead to the deadly dengue hemorrhagic fever. Here, we aim at characterizing the thermodynamic stability of DEN envelope protein domain III (ED3) during its evolution, and we report a structural analysis of DEN4wt ED3 combined with a systematic mutational analysis of residues 310 and 387 (Fig 1).

2 Experiments

Synthetic genes encoding the ED3 sequences were cloned into a pET15b vector (Novagen) . The sequences of DEN3wt and DEN4wt ED3 were retrieved from Uni-Prot (ID P27915.1). Mutations at the 310/387 sites were introduced by site directed mutagenesis using a Quik Change protocol (Stratagene, USA). The proteins were overexpressed in *E. coli* JM109 (DE3) PLysS, purified by reverse phase HPLC, and lyophilized. The melting temperatures were measured by monitoring the melting curves of the secondary structure content using a Jasco J820 CD spectrophotometer. The X-ray diffraction data were recorded from single crystals at the Photon Factory, and the structures were determined by molecular replacement.

3 Results and Discussion

Molecular modeling based on our previously determined structure of DEN3 wt ED3 [1] and the present DEN4 wt ED3 [2,3] structures indicated that the side-chains of residues 310/387, which are Val/Ile and Met/Leu in DEN3wt and DEN4wt, respectively, could be structurally compensated, and that a “size switch type repacking” (Fig 2) might have occurred at these sites during the evolution of DEN into its four serotypes. This was experimentally confirmed by a 10ºC and 5ºC decrease in the thermal stability of DEN3 ED3 variants with Met^{310}/Ile^{387} and Val^{310}/Leu^{387}, respectively, whereas the variant with Met^{310}/Leu^{387}, which contains a double mutation, had the same stability as the wild type DEN3. Namely, the $Met^{310}Val$ mutation should have preceded the $Leu^{387}Ile$ mutation in order to maintain the tight internal packing of ED3 and thus its thermodynamic stability.

This view was confirmed by a phylogenetic reconstruction indicating that a common DEN ancestor would have Met^{310}/Leu^{387}, and the intermediate node protein, Val^{310}/Leu^{387}, which then mutated to the Val^{310}/Ile^{387} pair found in the present DEN3. The hypothesis was further confirmed by the observation that all of the present DEN viruses exhibit only stabilizing amino acid pairs at the 310/387 sites.

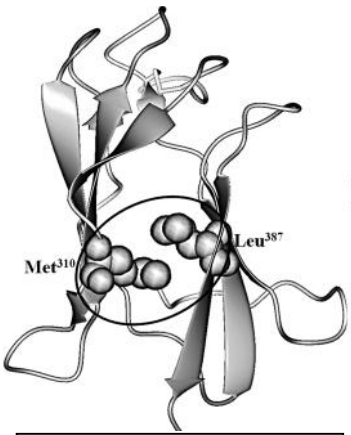

Fig 1: Ribbon model of DEN4wt. Residues 310 and 387 are encircled.

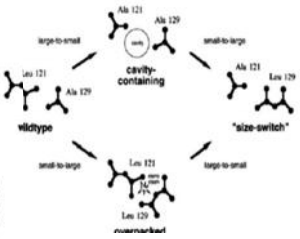

Fig 2: Schematics of size switch type repacking at positions 310 and 387.

References

[1] Elahi M., et al Proteins 81, 1090-1095 (2013).

[2] Elahi M, et al Biochim Biophys Acta. Proteins and Proteomics 1844(3), 585-92 (2014).

[3] The coordinates and structure factors of DEN4 ED3 have been deposited in the Protein Data Bank (PDB) under the accession number 3WE1.