5A/17A/ 2013G133

Crystal structure of dengue-3 envelope protein domain III suggests possible molecular mechanisms for serospecificity

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1 Introduction

Dengue virus is a major public health problem in south-east Asia including some isolated outbreak in the south of Japan. The viruses are classified into four serotypes (DEN1–DEN4), which have high sequence and structural similarities. However, because of minute differences in local structural conformation on the viral surfaces, antibodies produced against one serotype bind other ones with decreased affinity, thereby failing to neutralize the virus. This is thought to be responsible for the progression of dengue pathogenesis from simple dengue fever to the deadly dengue hemorrhagic fever upon infection by multiple serotypes. Here, we report a 1.7Å crystal structure of a recombinant dengue-3 envelope protein domain III (ED3), which contains most of the putative epitopes [1,2].

2 Experiments

The ED3 protein sequence was taken from the Uni-Prot database (ID P27915.1). The nucleotide sequence was synthesized and cloned into a pET15b expression vector (Novagen). The protein was over-expressed in a JM109 E.coli, and purified using Nickel resin column and reverse phase HPLC, and lyophilized. A crystallization stock solution was prepared by dissolving the lyophilized protein in 15 mM Tris-HCl pH 7.0. Crystals used for diffraction were developed at 20C in 30% PEG4000, 0.2M lithium sulfate, and 0.1MTris-HCl pH 8.5. The X-ray diffraction data were recorded from single crystals at the Photon Factory (Tsukuba, Japan), and the structures were determined by molecular replacement. The diffraction data were processed with the HKL2000 program package. The structure was solved by molecular replacement (MOLREP, CCP4i suite) using the structure of DEN1 ED3 (PDB: 3IRC) as molecular probes.

3 Results and Discussion

DEN3 ED3 crystal was a primitive monoclinic cell with space group P121 with two monomers per asymmetric unit. The structure was determined at 1.7Å resolution with a R-factor of 20% . The overall fold of DEN3 ED3 was very similar to other serotype's ED3 structures and contained ten β-strands arranged in two perpendicular planes forming a β-barrel like structure. Upon comparison of isolated ED3 with the full length E-protein ED3 structure, we found minute but definitive a local backbone deformation in the first β-strand, which contains the putative epitope-1. Further, a comparison with DEN-2 ED3 indicated a large structural change by as much as 4.0 A at Asp662, which is located in epitope-2. Furthermore, mutations also introduced changes in the electrostatic potential around the epitope regions (Figure 1). The molecular surface of epitope-1 in DEN1 was negatively charged but it was clearly positive in DEN3.

In conclusion, these minute structural and surface property changes observed in the high resolution ED3 structure represent potential determinants for serospecificity and epitope recognition by antibodies. Altogether, this study suggest that comparison of ED3 structures from different serotypes and crystallized under different conditions could provide insight into the mechanism of antibody recognition and thereby might open the way to the design of novel therapeutics.

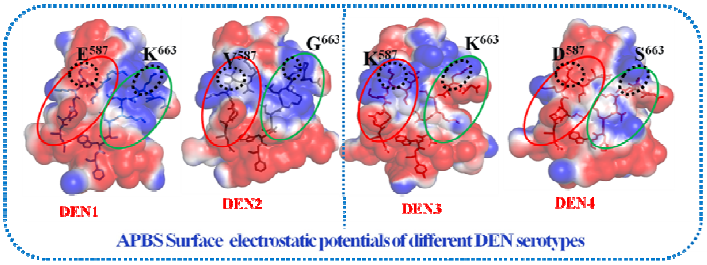

Figure 1: Surface model of ED3 DEN1~DEN4. The epitope regions are indicated by red and green circles. DEN3 structure was solved in this project (PDB:3VTT). Other serotypes Ed3 structures are retrieved from PBD

References

[1] Elahi Montasir et al., *PROTEINS,* (2013) **81**(6),1090-5.

[2] The coordinates of DEN3 ED3 have been deposited in the Protein Data Bank under the PDB entry code 3VTT.