3D Molecular Structure Analysis of NS2B/NS3 Proteases Derived from Dengue Virus and Zika Virus

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Introduction

Dengue virus most commonly known as DENVs is a single stranded RNA virus transmitted by *Aedes* mosquito and is the prominent cause of the dengue hemorrhagic fever (Bäck & Lundkvist, 2013). World Health Organization (WHO) estimates the number of annual incidence summing up to approximately 100 million infections with 500,000 people affected to dengue hemorrhagic fever would requiring hospitalization (Bäck & Lundkvist, 2013). While Zika virus or commonly called as ZIKV is one of the arboviruses transmitted by *Aedes* mosquito. It is a single-stranded RNA virus belongs to the genus *Flavivirus* of the *Flaviviridae* (Rawal et al, 2016). DENV and ZIKV are both mosquito-borne *Flavivirus* with similar transmission cycle, distribution throughout tropics and disease manifestations (Sharp et al, 2019).

To reach the maturation of DENV particles would require a proper cleavage of the viral polyprotein. Hence, the cleavage will includes the processing of eight of the thirteen substrate cleavage sites by dengue virus NS2B/NS3 protease and this is essential for maturation of the viral particle (Lin et al, 2017). DENV NS2B/NS3 is a protease that belongs to the chymotrypsin family. NS2B as the cofactor is required for the proper function of NS3 that eventually participates in substrate recognition (Lin et al, 2017). While ZIKV contains a positive-sense single-stranded RNA genome that encodes a single 3419 amino acid forming a polypeptide, which is then cleaved to form three structural proteins and seven non-structural proteins (Bowen et al, 2019). In the case of ZIKV NS2B/NS3, its NS3 contains the catalytic triad, and along with the transmembrane NS2B will form the NS2B/NS3 protease that is critical to the replication and survival of ZIKV (Bowen et al, 2019). Both are knowingly belong to the same genus of *flavivirus* and the identified NS2B/NS3 protease play a critical function in the development, replication and survival of the viral particle of both viruses. Hence, the study regarding the inhibitors of NS2B/ NS3 protease in both viruses is a promising target for drug development.

The main goal in this study is to gain different insight of the NS2B/NS3 proteases from both DENV and ZIKV by identifying the structure, and compare the similarity and differences between the structure. Analysis of the 3D structure of NS2B/NS3 proteases from both viruses conducted by visualization through a molecular graphic software. Further analysis can also be made

in terms of molecular stability and function by subjecting the structure to molecular docking simulation to compare the difference on how both NS2B/NS3 protease bind to a specific substrate. Molecular docking is a computational method to predict non-covalent binding of macromolecules specifically a receptor and a ligand efficiently (Trott & Olson, 2010).

Materials and Method

NCBI's Molecular Modeling Database (MMDB) based on the Protein Data Bank (PDB) maintains a comprehensive and up-to-date archive of protein structure similarities with Vector Alignment Search Tool (VAST) (Madej et al, 2014). VAST (**https://www.ncbi.nlm.nih.gov/Structure/VAST/vastsearch.html**) itself built and is integral part of National Center for Biotechnology Information (NCBI). In this research, VAST will be used alongside with the VAST+. VAST+ is the enhanced version of VAST which able to generate a report of the structure neighbours with the aim to find the largest set of pairs of matching macromolecules between two biological assemblies, characterize the match and compute instructions to visualize the structural similarity (Madej et al, 2014). In this research, comparative analysis was conducted between both proteases in terms of molecular structure specifically at the level of primary and secondary structure of the enzymes.

Full information regarding the proteases from both DENV and ZIKV were obtained from Protein Data Bank (PDB) under the name of Dengue Virus NS2B/NS3 Protease (PDB ID: *2FOM*; **https://www.rcsb.org/structure/2FOM**) and Zika virus NS2B/NS3 Protease (PDB ID: *5GXJ*; **http://www.rcsb.org/structure/5GXJ**). *2FOM* was subjected to search for similar structures by using VAST. The subsequent search will generate all matching molecules superposed. Hence, we eliminate the rest of the result and only consider the 5GXJ as we want to directly compare between both structures. Visualization of the 3D structure superposition and result of sequence alignment are all observed by using iCn3D a well-known web-based 3D structure viewer provided by NCBI.

Result and Discussion

It was clear that the replication of flaviviruses would require the correct processing of the polyprotein particularly by the NS3 protease. In overall, the NS3 protease domains of DENV adopt a chymotrypsin-like fold with two β-barrels with each formed by six β-strand alongside catalytic triad located at the cleft between two beta-barrels ***(Figure 1A)***. Here, the overall structure of DENV NS3 referred to the blue color strand while the NS2B indicated by purple color ***(Figure 1A).*** According to the previous study, the DENV NS2B/NS3 proteases structure, the electron density beyond NS2B residue 76 is discontinuous and thus suggesting that the C-terminal part of the cofactor may adopt multiple conformations in solution (Erbel et al, 2006). Through an observation, the structure of DENV and ZIKV NS2B/NS3 proteases

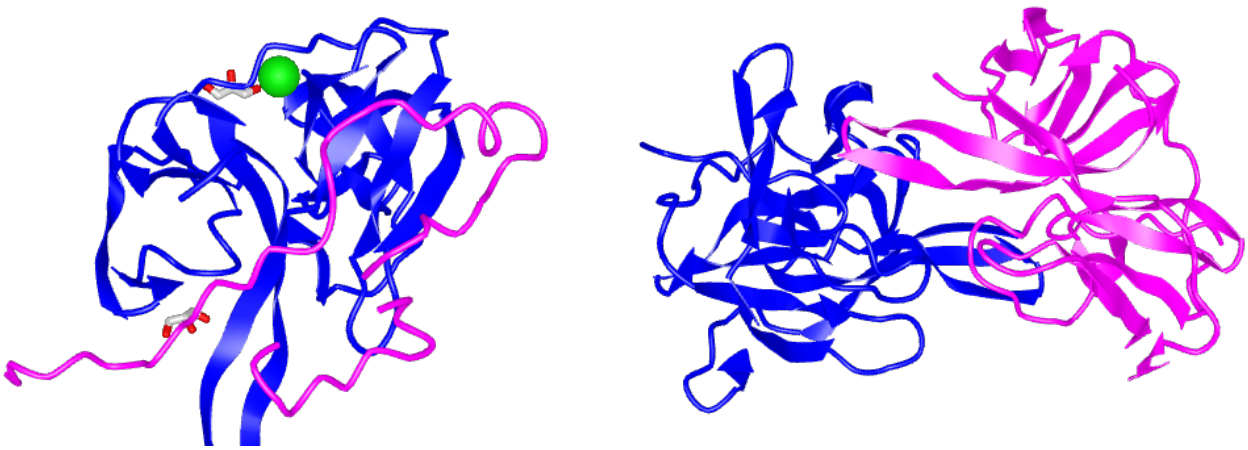

Figure 1A. *3D dimeric structure of DENV NS2B/NS3 Proteases (2FOM) (left);*

Figure 1B. *3D dimeric structure of ZIKV NS2B/NS3 Proteases (5GXJ) (right).*

are not quite similar despite various literature stated similarity between them. The structure of *5GXJ* itself have been published either under the PDB or NCBI. However, unlike *2FOM*, the literature regarding this structure is currently undergoing to be published. Therefore in this research, the *5GXJ* will be described briefly according to the information generated by NCBI's MMDB. Comparison of both structure shows the difference in terms of polyprotein specifically the length. For example, the polyprotein chain A of *2FOM* (purple color) refer to the NS2B has the length of 62 amino acids ***(Figure 1A),*** while the same polyprotein chain A of *5GXJ* has the length of 224 amino acids ***(Figure 1B).***

Aside of it, both *2FOM* and *5GXJ* have the same region of NS2B and peptidase, however both located at different position each other. For instance, in *2FOM*, NS2B is located in the polyprotein A chain while the subsequent peptidase is located inside the polyprotein B chain. While in *5GXJ*, as both chain have the same length of 224 amino acids, therefore the NS2B alongside with the peptidase are located within the same region of aforementioned polyprotein chains.

Sequence alignment was subjected to both structure in order to gain insight of similarity between two structures by using alignment and to visualize the 3D structure superposition. The ensuing alignment stated

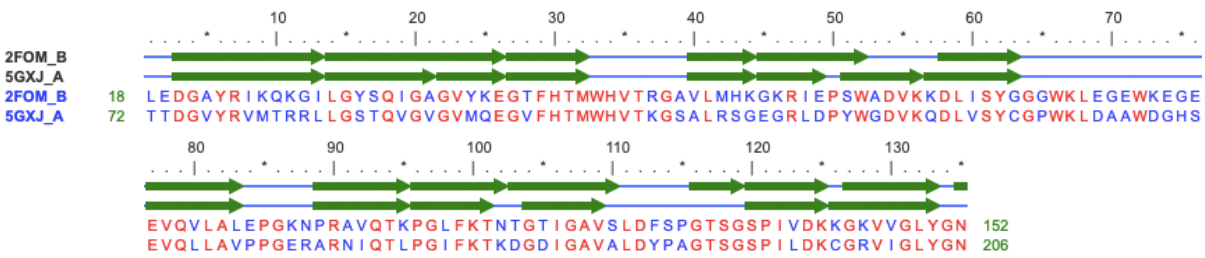

Figure 2. *Alignment result of DENV NS2B/NS3 protease (2FOM) and ZIKV NS2B/NS3 protease (5GXJ)*

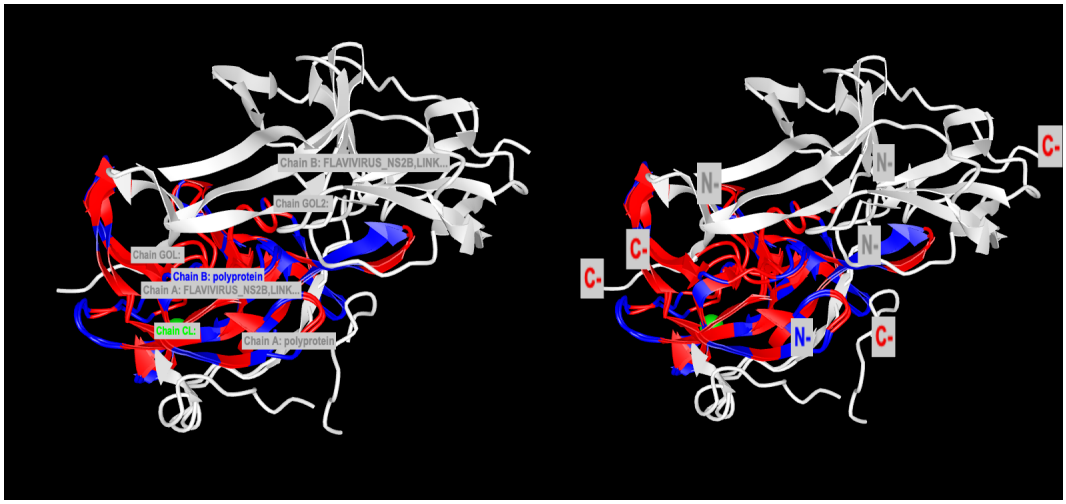

Figure 3A. *3D structure of the ensuing alignment 2FOM and 5GXJ with the addition of label chain.* ***Figure 3B.*** *Location of N- and C-terminal from the alignment of 2FOM and 5GXJ.*

as seen on ***Figure 2.*** Subsequent alignment only take the polyprotein chain B of *2FOM* and polyprotein chain A of *5GXJ* as the main template for the alignment process. Alignment result showed there are 60 mismatches of amino acids out of 135 that are subjected to the alignment process. Thus, making up the similarity only just 56%. 3D structure analysis showed the result of alignment as seen on ***Figure 3A*** and ***Figure 3B***. As we can see, the combination of blue and red color ribbon indicates the successful alignment of polyprotein B chain of *2FOM* with polyprotein A chain of *5GXJ*. Observation also showed the Polyprotein B chain of *5GXJ* are likely unaligned due to the difference in the length with the counterpart ***(Figure 3A).*** Hence, this possibly would be the answer why the similarity of both structure only just making up about 56%. The contribution of NS2B itself in the formation of an active protease in between the *flaviviridae* family differ substantially especially those observed with other cofactor- activated viral proteases (Erbel et al, 2006). However, in regards to the NS2B itself, it completes the substrate-binding site with its C-terminal region and create stability in both N- and C-terminal by creating additional β-strands (***Figure 3B***) (Erbel et al, 2006).

This study provides a 3D molecular structure overview of NS2B/NS3 derived from both DENV (*2FOM*) and ZIKV (*5GXJ*). In overall, both structure are not similar as from the sequence alignment, the rate of similarity is only just 56%. However, in terms of function both have NS2B that is pivotal for the activation of NS3 proteases. Hence, will allow proper cleavage and replication of viruses. However, lot of limitations are identified in this study. First, we only conduct a one on one comparison between NS2B/NS3 of DENV and ZIKV. This one on one comparison may cause bias in the analysis as different structures that came from the same viruses are available in the NCBI's MMDB. Second, indeed *2FOM* already previously studied since 2006 by Erbel et al, however, the *5GXJ* from ZIKV

that was used in this study have no publications yet and therefore, the ensuing analysis might be questionable. Third, we are not conducting molecular docking simulation to compare the difference on how both NS2B/NS3 protease bind to a specific substrate.

Conclusion

NS2B/NS3 proteases are the integral part in Flavivirus. Their replication would require the correct processing of their subsequent polyprotein by NS3 protease. In this case, NS2B is important as the activator of NS3. These studies only focus on the 3D molecular structure analysis of NS2B/NS3 derived from both viruses. As we refers to the limitations above, lot of studies might be elaborated in the future. Comparison between an NS2B/NS3 from DENV with several variant of NS2B/NS3 from ZIKV might be possible to gain insight the difference in terms of structure. Furthermore, molecular docking simulation might be a useful method to observe the difference in terms of the ability on how both NS2B/NS3 proteases bind to a specific substrate.

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