Impact of Temperature on Structural Changes in nsp2 and nsp3 of CHIKV: Molecular Dynamics Simulations

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Abstract

Authors have considered non-structural protease-2 (nsp2) and non-structural protease-3 (nsp3) of CHIKV for the investigation as they are involved in the replication of the virus and increases the infections in the human. CHIKV protease (nsp2), has a catalytic dyad (Cys¹⁰¹³/His¹⁰⁸³) that processes viral polyproteins. Further, it has also been suggested that the catalytic dyad is interchangeable to a serine (proximal) and hence making called non-papain-like cysteine protease. Similarly, nsp3 plays important role during replication of CHIKV genome. In this work, we have investigated the effect of temperature on the structural stability of nsp2 and nsp3 of CHIKV. Although, we did not get any regular patten for change in the RMSD and RMSF for the nsp3 of CHIKV and observed that RMSD for nsp3 of CHIKV is acceptable at all the temperature (300, 325 and 350 K) but the minimum RMSD values are obtained at 325 K. Similarly, no regular pattern is observed for RMSF trajectories of nsp3 of CHIKV at different temperature and minimum fluctuations in the position of amino-acids at 325 K. Further, the RMSD and RMSF trajectories for nsp2 of CHIKV are investigated and observed a regular pattern in the RMSD and RMSF trajectories generated from MD simulations on increasing the temperature. In this, RMSD values are minimum at 300 K while the fluctuation in the position of amino-acids are maximum.

1. Introduction

Chikungunya virus (CHIKV) is a mosquito-borne arboviral disease. CHIKV belongs to *Togaviridae* family and Alphavirus genus, consisting of enveloped, positive single-stranded RNA viruses. In humans, there is a rapid onset of CHIKV infection and typically cleared in 5-7 days. It occurs via bite of an infected *Aedes aegypti/albopictus*, replicates in skin and from there it disseminates to liver and joints via blood. A rapid onset of disease with no pro-dermal phase, following an incubation period of 2-4 days.[1–3] CHIKV enters into the target cells via endocytosis. Upon endocytosis, endosomes undergo conformational change in acidic environments in viral envelope and exposes E1 peptide, mediating virus-host cell membrane fusion. This allows release of viral genome. The viral mRNA translates precursor non-structural proteins (nsps) and undergo cleavage to generate nsp1-nsp4. These nsps such as nsp1 (negative strand viral RNA synthesis), nsp2 (RNA helicase), nsp3 (replicase unit), and nsp4 (viral RNA polymerase).[4–7] Nsps form the replication complex and form RNA intermediates to synthesize sub-genomic (26S) and genomic (49S) RNAs. Following this, the capsid and glycoproteins are generated, leading to viral assembly by recruiting membrane-associated glycoproteins. It consists of capsid (C) protein that forms nucleocapsid. The envelope of viral particle consists of E1 and E2 transmembrane glycoproteins that arrange into 80 spikes with three E1/2 heterodimer. The E1 and E2 facilitates membrane fusion and binding to the host cell, respectively. Its genome consists of two ORFs that are flanked by 5' and 3' UTRs and separated by non-coding intergenic region. The ORF1 and ORF2 encodes for nsp1-4 and structural proteins such as capsid, glycoprotein, virion channel, etc., respectively.[4, 8–11] CHIKV protease (nsp2), has a catalytic dyad (Cys¹⁰¹³/His¹⁰⁸³) that processes viral polyproteins. Further, it has also been suggested that the catalytic dyad is interchangeable to a serine (proximal) and hence making called non-papain-like cysteine protease. Similarly, nsp3 plays important role during replication of CHIKV genome.[2, 12, 13]

Molecular dynamics (MD) simulations is a time-saving, computational approach to perform molecular docking and provides the energy for complex formation between the receptor and a small molecule. Temperature-dependent MD simulation can be performed to understand the impact of temperature on the activity of non-structural protease of CHIKV. Upon screening of a small molecule against the nsp of CHIKV, at fluctuating temperatures, gives the binding energy for the complex formation that may vary with temperature. The temperature-dependent MD simulations would give different trajectories that can be analyzed to understand the change in the nsp.[9, 11, 14–17] MD simulation is a computer simulation-based approach that analyze physical movements of atoms, molecules and biomacromolecules such as protein, DNA and more. It's an important and effective approach to study physical and chemical changes of small molecules. MD simulations combining with Newton's second law of motion to obtain an output of MD simulation trajectories, which are snapshots of simulated molecular system, representing atomic coordinates at specific time periods. The trajectories are generated by Newton's second law of motion for time evaluation of n interacting particles.[9, 14, 18–20]

We all witness to various viral infection; have threatened the humanity. Researchers are working on the inhibition of the virus through experimental work as well as computational methodologies to get the effective or promising inhibitors. Authors have considered non-structural protease-2 (nsp2) and non-structural protease-3 (nsp3) of CHIKV for the investigation as they are involved in the replication of the virus and increases the infections in the human. In this work, the impact of temperature on non-structural protease-2 and non-structural protease-3 of CHIKV has been investigated using the molecular dynamics simulations for 100 ns at 300, 325 and 350 K.

2. Computational Calculations

2.1 Preparation of nsp2 and nsp3

Crystal structure for the nsp2 and nsp3 of CHIKV has been taken from the RCSB. The PDB ID for the nsp3 and nsp2 of CHIKV are 3GPO and 3TRK with resolution of 2.397 and 1.9 Å. These proteases are prepared before running the molecular dynamics simulations at different temperature using the pymol and UCSF-1.13.1 software. The software is used to remove the present ligands, cofactors and solvent molecules. Further, add the missing atoms as well as the incomplete chain and assign charges to the atoms in the pdb files as mentioned above.[6, 10, 20–23].

2.2 Molecular dynamics (MD) simulations

MD simulations of nsp2 of np3 of CHIKV are performed using AMBER 18 suite. All the atoms present in the nsp2 and nsp3 are prepared using generalized Amber Force Field (GAFF) and then the water molecules were modelled on applying the TIP3P, a force field. The details of the methodology can be found in our previous research work on the various non-structural protease of CHIKV.[9, 10, 20, 23, 24] MD simulations of nsp2 and nsp3 of CHIKV are performed for 100 ns at 300, 325 and 350 K. To understand the change in the structural stability and flexibility, two trajectories, named root mean square deviation (RMSD) and root mean square fluctuation (RMSF) are generated.

3. Result And Discussion

RMSD is used to explain the motion of the atoms of the protease with time in nanoseconds. It provided the information for the stability on changing time at a particular temperature in terms of deviation of the position of the atoms. RMSD is a quantitative scale to investigate the similarity or dissimilarity of the protease on change the temperature. It may explain the folding or unfolding of the protease with change in temperature. Higher values of the RMSD and the RMSF may indicate the flexibility or the fluctuation of the protease on changing the temperature. RMSD and RMSF are used to differentiate the flexible from the rigid structure of the nsp of CHIKV.

3.1 RMSD analysis of nsp2 and nsp3 of CHIKV

In this work, the divergence is not seen from the beginning and occurs after some time and shows the equilibration is done satisfactorily. MD simulations of nsp3 of CHIKV is performed at 300, 325 and 350 K as in Figure 1. From the RMSD trajectories, it can be easily seen that RMSD for nsp3 of CHIKV value at all temperature is acceptable. Actually, no regular pattern has been observed for the nsp3 of CHIKV on varying the temperature as the RMSD values are higher at 300 and 350 K than at the 325 K. It indicates that at 325 K, there is less deviation means the structure of the nsp3 of CHIKV is compact; while the deviation is maximum at 300 K and has less compact structure. This information suggest that stability of the protease is maximum at 325 K.

Then, RMSD trajectories are generated from MD simulations of nsp2 of CHIKV as in Figure 2. We have obtained a patten for the values of RMSD of nsp2 of CHIKV on increasing the temperature. The interesting part if on increasing the temperature, the RMSD values for the nsp3 of CHIKV increases and at 350 K, the RMSD values increases a lot and crossed 3 Å and not acceptable. Although, the value of deviation at 325 are acceptable but still are much higher than at 300 K. So, it can be concluded that nsp2 of CHIKV at 300 K has a compact structure and stable.

3.2 RMSF analysis of nsp2 and nsp3 of CHIKV

Based on satisfactory equilibration, the structure of the protease fluctuated from the stable conformation of the protease of interest. This is also used to understand the stability of the protease based on the fluctuation in the amino acids. Based on the Figure 3, it can be understood that there is no regular patten in the fluctuation of amino-acids on changing the temperature. The minimum fluctuations in the amino-acids of nsp3 of CHIKV is observed at 325 K. It corroborates with information obtained from the RMSD trajectories of nsp3 of CHIKV at different temperature (300, 325 and 350 K).

Now, trajectories of RMSF for nsp2 of CHIKV are generated from the MD simulations as in Figure 4. A regular pattern is observed in the fluctuation of amino-acids of nsp2 of CHIKV on increasing the temperature. Herein, the maximum fluctuations are observed at 300 K for the residues 60-70, 90-120, 140-200, 230-265 and 270-290. With increase in temperature, the fluctuation in the amino-acids decreases.

4. Conclusion

In the present work, we have investigated the effect of temperature on the structural stability of nsp2 and nsp3 of CHIKV. We did not get any regular pattern for change in the RMSD and RMSF for the nsp3 of CHIKV and observed that RMSD for nsp3 of CHIKV is acceptable at all the temperature (300, 325 and 350 K) but the minimum RMSD values are obtained at 325 K. Similarly, no regular pattern is observed for RMSF trajectories of nsp3 of CHIKV at different temperature and minimum fluctuations in the position of amino-acids at 325 K. Further, the RMSD and RMSF trajectories for nsp2 of CHIKV are investigated and observed a regular pattern in the RMSD and RMSF trajectories generated from MD simulations on increasing the temperature. In this, RMSD values are minimum at 300 K while the fluctuation in the position of amino-acids are maximum.

Declarations

Author Contributions: All authors have read and approved the version of the manuscript. Experiments are performed by [Durgesh Kumar] and [Mahendra Kumar Meena]. The manuscript was written by [Vinod Kumar] and [Prashant Singh].

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Figures

Figure 1

RMSD plot of nsp3 of CHIKV at 300, 325 and 350 K.

Figure 2

RMSD plot of nsp2 of CHIKV at 300, 325 and 350 K.

Figure 3

RMSF plot of nsp3 of CHIKV at 300, 325 and 350 K.

Figure 4

RMSF plot of nsp2 of CHIKV at 300, 325 and 350 K.

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