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Topical Perspectives

Interaction of monomeric Ebola VP40 protein with a plasma membrane: A coarse-grained molecular dynamics (CGMD) simulation study

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ABSTRACT

Ebola virus is a lipid-enveloped filamentous virus that affects human and non-human primates and consists of several types of protein: nucleoprotein, VP30, VP35, L protein, VP40, VP24, and transmembrane glycoprotein. Among the Ebola virus proteins, its matrix protein VP40 is abundantly expressed during infection and plays a number of critical roles in oligomerization, budding and egress from the host cell. VP40 exists predominantly as a monomer at the inner leaflet of the plasma membrane, and has been suggested to interact with negatively charged lipids such as phosphatidylinositol 4,5-bisphosphate (PIP_2) and phosphatidylserine (PS) via its cationic patch. The hydrophobic loop at the C-terminal domain has also been shown to be important in the interaction between the VP40 and the membrane. However, details of the molecular mechanisms underpinning their interactions are not fully understood. This study aimed at investigating the effects of mutation in the cationic patch and hydrophobic loop on the interaction between the VP40 monomer and the plasma membrane using coarse-grained molecular dynamics simulation (CGMD). Our simulations revealed that the interaction between VP40 and the plasma membrane is mediated by the cationic patch residues. This led to the clustering of PIP_2 around the protein in the inner leaflet as a result of interactions between some cationic residues including R52, K127, K221, K224, K225, K256, K270, K274, K275 and K279 and PIP_2 lipids via electrostatic interactions. Mutation of the cationic patch or hydrophobic loop amino acids caused the protein to bind at the inner leaflet of the plasma membrane in a different orientation, where no significant clustering of PIP_2 was observed around the mutated protein. This study provides basic understanding of the interaction of the VP40 monomer and its mutants with the plasma membrane.

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1. Introduction

Ebola virus is a lipid-enveloped filamentous virus that affects human and non-human primates to have Ebola virus disease, causing severe haemorrhagic fever with case fatality rates of up to 90% [1,2]. The virus contains a negative-sense RNA genome that encodes seven proteins: nucleoprotein, VP30, VP35, and L protein constituting the nucleocapsid (NC); transmembrane glycoprotein, which accounts for the entry of virions into the host cell; VP24, a minor protein crucial for the assembly of NC; and VP40, the viral matrix protein that regulates viral budding and NC recruitment, and virus structure and stability [3].

VP40 can induce virus-like particles (VLP) formation and bind to cellular membranes in a hydrophobic manner [4]. VP40 crystal structure resolved at 2.0 Å resolution reveals two domains with unique folds [5]: the C-terminal domain has large cationic patches that may involve in the interaction with lipid bilayers for membrane binding, while the N-terminal domain is important for VP40 oligomerization [3,5–7]. VP40 can undergo major structural rearrangement once at the plasma membrane and oligomerize into hexameric structures [8,9].

VP40 has been associated with several types of lipid like phosphatidylserine (PS), which regulates the assembly of virus matrix protein [10]; and phosphatidylinositol 4,5-bisphosphate (PIP_2), which is important for the assembly of the VP40 at the plasma membrane for subsequent VLP formation [11]. The VP40 hexamer

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has been shown previously to enhance the clustering of PIP_2 through electrostatic interaction with Lys residues of the cationic patch [12]. However, there is little information on the interaction between the VP40 monomer and the plasma membrane, which is the focus of this study. Understanding the dynamics of the interaction between different oligomeric states of VP40 proteins may help in formulating pharmacological strategies for inhibiting the budding of the Ebola virus from the plasma membrane.

Despite of the potency of the virus via the VP40 matrix protein, study on the monomer structure of the protein using molecular dynamics (MD) simulation is lacking. Although VP40 can be presented in dimer, hexamer and other larger oligomeric forms, VP40 proteins on the membrane are predominantly VP40 monomers, suggesting that monomeric VP40 is recruited from the cytosol to serve as building block for oligomerization [13]. Furthermore, mutation of VP40 hydrophobic loop residues has been shown to abrogate plasma membrane localization while mutation of some residues at the cationic patch reduce the interaction of the protein with the plasma membrane [7,8].

This study employed coarse-grained molecular dynamics (CGMD) simulation approach to gain insight on the binding mode and key residues interactions of the Ebola VP40 monomer and its mutants with the plasma membrane. CGMD was used to overcome limited timescales of atomistic-detail approaches [14], and the use of coarse-grained (CG) particles simplify the complex energy landscape of the simulation [15,16]. CG models have been used to investigate interactions between protein and lipid, such as PTEN protein and membrane [17], VP40 hexamer and membrane [12], monotopic membrane enzyme and lipid bilayer [18], and transmembrane self-assembly in lipid bilayer glycophorin A [19] and influenza M2 channel [20].

The results presented here show the basic interaction of VP40 monomer and its mutants with the plasma membrane, in which positively charged residues and membrane association lead to significant PIP_2 clustering. However, upon mutation of key residues of the cationic patch or the hydrophobic loop, no significant PIP_2 clustering was formed, and the VP40 monomer bound in different orientation compared to the wild type (WT). This suggests that either region could potentially be targeted for small molecules to block VP40 membrane association. Overall, our study of the VP40 monomer using CGMD gives some insight on the dynamic interaction of the VP40 monomer with the plasma membrane.

2. Materials and methods

2.1. VP40 monomer and mutant models

The VP40 monomer x-ray crystal structure was obtained from the Protein Data Bank (PDB) [PDB ID: 1ES6] [5]. The structure was visualized using VMD [21], and missing residues were inserted using MODELLER version 9.17 [22]. Then, energy minimization was performed on the complete structure using GROMACS 5.1.4 [23].

To examine the effects of mutation of certain amino acid residues on the interaction of the VP40 monomer with the plasma membrane, two mutant models were generated using PyMOL mutation tool [24]: VP40 C-terminal domain hydrophobic loop amino acid residues L213, I293, L295, and V298 were mutated into Ala (mutant A); and four residues of the C-terminal domain cationic patch, namely K221, K236, K274, and K275 were mutated into Glu, a negatively charged amino acid (mutant B).

2.2. Plasma membrane model

CG structure of the plasma membrane was generated using CHARMM-GUI web Martini Maker plug-in [25]. It consisted of six types of lipids: POPE, POPC, PIP_2, PSM, POPS, and CHOL. Heterogenous lipids were used in order to mimic the condition of real human plasma membrane. The proportion of lipids (POPC:POPE:PSM:POPS:PIP_2:CHOL) in the modelled membrane was (41:8:23:4:4:20) in the extracellular leaflet and (11:37:5:16:10:21) in the intracellular leaflet, based on GC et al. [12]. It comprised 400 lipid molecules, large enough to accommodate the protein inside the bilayer. The generated plasma membrane was equilibrated through a six-step equilibration process in CHARMM-GUI.

2.3. System and simulation setup

Four heavy atoms were mapped onto a single interaction site (bead) except for residues with aromatic side [26,27]. CG structures of the VP40 monomer and its mutants were generated by converting the atomistic structures of the protein (WT, mutant A and mutant B) using the martinize.py script from the Martini website utilizing spatial restraint for the conversion [26–28]. CGMD simulations were performed using GROMACS version 5.1.4 with Martini 2.2 force field [23]. Each CG structure was combined independently with the plasma membrane in a $100 \times 100 \times 150$ Å simulation box. The protein was positioned at ~25 Å from the lipid headgroup with the amino acid residues of interest, the cationic patch and hydrophobic loop residues facing the intracellular leaflet of the plasma membrane (Fig. 1). The initial distance between the centre of mass (COM) of the protein and the membrane was 70 Å. Then, water was added, along with the appropriate number of counterions to neutralize its net charge. Water found inside the bilayer was removed using the killwater.pl script [29].

The system was then energy-minimized for up to 1000 steps using the steepest descent and subsequently equilibrated for 20 ns by restraining the protein position before the production run. Pressure during the equilibration run was maintained at 1 bar using the Berendsen barostat. For the simulation setup, a time step of 20 fs was used and the simulation was run first for 1 μs, and then extended to 5 μs in triplicates. Verlet neighbour scheme was used with a straight cut-off of 11 Å while ring systems and stiff bonds were controlled using the LINCS algorithm. Coulomb interactions were treated using a reaction-field. The simulation was run using the Parrinello-Rahman barostat, where the pressure coupling between protein and lipid groups was semi-isotropic with a compressibility of 3×10^{-4} bar^{-1}. The temperature was set at 303.15 K and velocity rescale algorithm was used for the temperature coupling. Analysis of the trajectories, visualization was performed using VMD [21], and the interaction of the protein with the

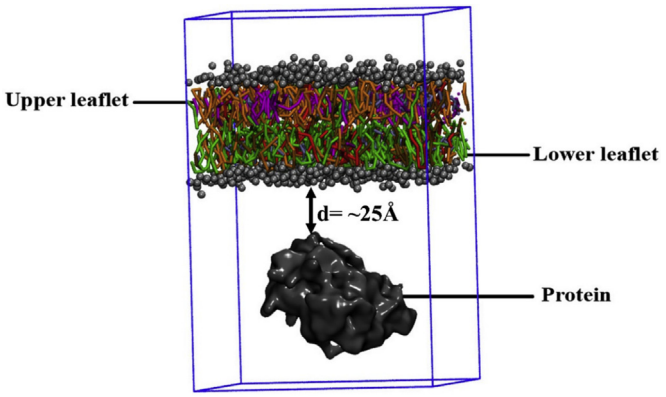

Fig. 1. A snapshot of simulation system consists of a monomeric VP40 protein and a membrane in a simulation box with the amino acid residues of interest facing the intracellular leaflet of the bilayer. Water and ion molecules are not included for clarity.

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