Stability, orientation and position preference of the stem region (residues 689-703) in Hepatitis C Virus (HCV) envelope glycoprotein E2: a molecular dynamics study [version 1; peer review: 3 approved with reservations]

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Abstract
Envelope glycoproteins of Hepatitis C Virus (HCV) play an important role in the virus assembly and initial entry into host cells. Conserved charged residues of the E2 transmembrane (TM) domain were shown to be responsible for the heterodimerization with envelope glycoprotein E1. Despite intensive research on both envelope glycoproteins, the structural information is still not fully understood. Recent findings have revealed that the stem (ST) region of E2 also functions in the initial stage of the viral life cycle. We have previously shown the effect of the conserved charged residues on the TM helix monomer of E2. Here, we extended the model of the TM domain by adding the adjacent ST segment. Explicit molecular dynamics simulations were performed for the E2 amphiphilic segment of the ST region connected to the putative TM domain (residues 683-746). Structural conformation and behavior are studied and compared with the nuclear magnetic resonance (NMR)-derived segment of E2 (2KQZ.pdb). We observed that the central helix of the ST region (residues 689 - 703) remained stable as a helix in-plane to the lipid bilayer. Furthermore, the TM domain appeared to provide minimal contribution to the structural stability of the amphipathic region. This study also provides insight into the orientation and positional preferences of the ST segment with respect to the membrane lipid bilayer interface.
Introduction

Envelope glycoproteins E1 and E2 are essential for the initial binding and internalization of Hepatitis C Virus (HCV) into the host cells. Both glycoproteins have been shown to interact as a non-covalent heterodimer during biosynthesis\(^6\). Several conserved charged residues located in the TM domains of E1 and E2 were shown to function not only as membrane anchors, but were also essential for dimerization, endoplasmic reticulum retention and viral envelope formation\(^1\).

In our previous studies, we demonstrated the unfolding behavior of the E2 TM helix monomer was attributed to the charged Asp728 and involved the formation of a salt bridge with the Lys of the E1 envelope glycoprotein\(^5\). The ion-pair interaction of the E1–E2 heterodimer was captured in the molecular dynamics (MD) simulations based on the model that placed the charged Asp and Lys at the helix-helix interface\(^4\).

E2 envelope glycoprotein is known to be required for interactions with cellular receptors involved in endocytosis and membrane fusion\(^1\). E2 is composed of domain I-III, followed by the ST region and the TM domain. Recent studies based on circular dichroism (CD) and nuclear magnetic resonance (NMR) spectroscopy revealed that the soluble region located adjacent to the TM domain of E2 was involved in the initial virus entry. This highly conserved ST region was showed to fold as a helix upon membrane binding\(^2\).

In this work, we carried out MD simulations for three E2 structures: (1) a model generated by the I-Tasser server, (2) an ideal helix model and (3) an NMR derived structure of the E2 segment (2KZQ.pdb). The first two models include the TM domain of E2 but the TM domain is not present in the NMR structure. We observed consistent structural stability in the ST region amphiphilic segment (residue 689–703) across all simulations suggesting that the contribution of the TM domain to the segment structure stability is minimal. In addition, we demonstrated the orientation and positional preferences of this amphiphilic segment.

Methods

Input structure preparation

The protein sequence of HCV E2 genotype 1a (H77 strain) (Uniprot ID P27958) used to prepare the models for MD simulations was obtained from UniProtKB/Swiss-Prot database (www.uniprot.org)\(^9\). The following is the sequence segment of HCV E2 used to prepare the first model for this work, referred to as an ideal helix model:

\[
\text{683PALSGL} \quad 690\text{ILHQNI} DV \quad 700\text{QLYGVGS}I \quad 710\text{ASWAIK} \quad 720\text{KV} YV \quad 730\text{VLLL} L \quad \text{LAD}A \quad 740\text{RVCSCWL} \quad MML \quad 748\text{LISQAEA}. 
\]

The ideal helix model was generated using Pymol (http://pymol.sourceforge.net) by orienting the TM segment (residue 683–714) perpendicular to the TM domain (residues 715–746). The protein structure images in this work were prepared with the Pymol program. The I-Tasser webservice\(^1\) was used to generate the second model. The complete sequence of E2 was submitted to the I-Tasser server. Five models were generated and the model with a low root mean square deviation (RMSD) with the available NMR structure (2KZQ.pdb) and consisting of a helical TM domain was selected. Only the same segment as examined with the ideal-helix model was used for further MD simulations. The third model studied was the NMR-derived structure of E2 (PDBID: 2KZQ) that was obtained from the Protein Databank (PDB)\(^1\). This E2 protein segment was based on the HCV genotype 2a (JFH-1) (Uniprot ID Q99IB8)\(^4\).

System preparation

Pre-equilibrated dipalmitoylphosphatidylycholine (DPPC) lipid bilayer was retrieved from the web of Prof. Tieleman (http://moose.bio.ucalgary.ca/). Peptide orientation in DPPC lipid bilayer was done by aligning hydrophobic belt of the peptide, parallel to the membrane plane using LAMBADA\(^1\). The optimal number of overlapping lipid molecules was subsequently calculated and removed followed by lipid expansion (inflation) and alternating twenty steps of deflation and energy minimization to allow the peptide to be embedded within the bilayer using inflateGRO\(^2\). A short 100 ps energy minimization was employed to relax possible steric conflicts. Ions and counter ions were added to neutralize the system followed by 20 ns position-restrained simulation allowing the bilayer to re-equilibrate around the protein. Production MD simulations were carried out for 100 ns in the I-Tasser model, and 20 ns for both the ideal helix model and NMR structure (2KZQ.pdb).

MD simulations

The DPPC lipid bilayer interactions were described using the Berger force-field parameters\(^1\). The TM helices were modeled with the united atom force-field GROMOS96 53a6\(^1\). Simulations were performed with the Gromacs 4.5.5 package\(^1\) using 2-fs time steps. Periodic boundary conditions were used in all directions. Bonds to hydrogen atoms were constrained using the LINCS algorithms\(^7\). For the short-range van der Waals interactions, a cut-off distance of 1.0 nm was used. The long-range electrostatic interactions were treated using the particle mesh Ewald (PME) method with a grid spacing of 0.12 nm and cubic interpolation. The non-bonded pair list was generated every 10 steps with a cut-off of 1.0 nm. Water, lipid and peptide systems were coupled separately to temperature baths with 323 K for the DPPC using the Berendsen algorithm with a time constant of \(\tau_T = 0.1 \text{ ps}\). To maintain constant pressure, semi-isotropic coupling was employed separately for the lateral and for the normal directions with Berendsen weak coupling and a \(\tau_p = 1 \text{ ps}\) time constant. The compressibility was set to \(4.5 \times 10^{-5} \text{ bar}^{-1}\).

Analyses of the trajectories were primarily performed with tools included in the Gromacs 4.5.5 suite\(^1\). RMSD analyses were based on the coordinates of all atoms of the peptides. The bilayer thickness was measured by averaging the distances between lipid headgroups in the upper and lower leaflets of the lipid membrane with the tool GridMAT-MD\(^9\).

Results and discussion

Stability of the amphiphilic region

A stable helical conformation of the E2 ST region (residue 689–703) was consistently observed with some uncharacteristic spikes in the early stages and towards the end of the MD simulation of I-Tasser model and the NMR derived structure (2KZQ.pdb), respectively. RMSD of this amphiphilic segment was also consistently observed to be progressing within the commonly accepted 2 Å range for the ideal helix and NMR structures throughout the simulations. On the other hand, the I-Tasser model showed subtly higher RMSD progression over the simulation time (Figure 1, Figure 2 and Figure 3a, 3b).
Figure 5. Supplementary Figure 1.

Connor contribution of lipid to structural stability modulation and are in good agreement with the hypothesized lipid and/or protein contribution to structural stability. The higher RMSD value observed in the I-Tasser model simulation was well anticipated and was mainly attributed to the relative positioning of the ST region, which was sandwiched between lipid leaflets, forcing the segment to reorganize its structural conformation and having only a minimal effect on the helical integrity of the secondary structure. Examining this reorganization further by monitoring the distance of the amphiphilic segment to lipid leaflets led to another interesting observation described in the next section of this article.

Orientation and positional preference of the amphipathic segment

Monitoring the movement of the amphiphilic segment during the simulations led to another interesting observation. Segment of residues 689–703 in the I-Tasser model appeared to move towards the hydrophobic core of the lipid bilayer as depicted by steady progression in the distance to both the upper and lower lipid leaflets depicted in Figure 4. This reorganization is surprisingly interesting because we would have previously assumed that the amphiphilic region exposed to the solvent would hold the structure steady, despite some part of the amphiphilic region being initially vertically positioned in the hydrophobic core of the lipid (Supplementary Figure 1). In addition, given the amphiphilic nature of the residues in this segment, one could postulate that the residues would remain at this position. However, over the period of the simulation the segment reoriented by moving away from the lipid leaflets, while at the same time retaining structural integrity. The structural stability of the segment, as discussed in the previous section of this article, is attributed to the amphiphilic nature of the residues but this does not explain the movement towards the hydrophobic core of the lipid. The systematically orchestrated movement towards the hydrophobic core of the lipid leaflets indicates a strong orientation preference of the amphipathic segment, which in this specific case was parallel to the lipid leaflets. We then monitored the segment movement relative to the lipid leaflets with the other two simulations (the ideal helix model and the 2KZQ structure). The segment was initially positioned horizontally to the lipid leaflets. The results showed that the distance of the amphiphilic segment in both simulations was consistently within 4 Å to the lipid phosphate head group throughout the simulation (Figure 5). These data further clarify the orientation preference of the amphipathic segment with respect to the lipid leaflets and suggest that the residues are positioned in the membrane interface in a very stable manner. Interestingly, Albecka et al. speculated that these residues could have an in-plane topology or orientation and suggested that the ST region would ideally be positioned in the membrane interface, which is again in agreement with our data. We have described the behavior and provided a detailed insight into the dynamics of this amphipathic segment in a lipid bilayer environment.

Conclusion

In this study, the atomistic MD simulations provide insightful structural data for the E2 segment. The amphiphilic segment of E2 was able to remain as a stable helix in a lipid bilayer environment even without the respective TM domain. The results also revealed the orientation and positional preferences of the amphiphilic segment in relation to the water-bilayer interface that further clarify speculations from experimental studies.

Figure 2. Molecular dynamics simulation of E2 I-Tasser model.

Both amphiphilic region (segment 689–703) and TM domain located in the hydrophobic core of the bilayer. Length of residues 689–703 is plotted in red, root mean square deviation of the same residues with respect to the starting structure is plotted in black.

Secondary structure stability observed in these three contrasting simulation systems can be attributed to the amphiphilic nature of the residues allowing the helix to retain its structure on both the hydrophobic core of the lipid bilayer and the hydrophilic environment of the solvent. This amphiphilic characteristic of the residues has been discussed and described to great extent by Albecka et al. in a previous study. Interestingly, the presence of the TM domain does not appear to contribute significantly to the helix stability of the amphipilic region.

The helical length of this region does not vary much between the I-Tasser and the ideal-helix models (Figure 2 and Figure 3a), which include both the ST region and TM domain, compared with the 2KZQ.pdb (Figure 3b) which does not include the TM domain. This observation suggests that lipid-peptide interactions play a larger role in stabilizing the secondary structure of this amphipathic segment compared with the TM domain. These data provide evidence of the contribution of lipid to structural stability modulation and are in good agreement with the hypothesized lipid and/or protein contribution to structural stability. The higher RMSD value observed in the I-Tasser model simulation was well anticipated and was mainly attributed to the relative positioning of the ST region, which was sandwiched in between lipid leaflets, forcing the segment to reorganize its structural conformation and having only a minimal effect on the helical integrity of the secondary structure. Examining this reorganization further by monitoring the distance of the amphiphilic segment to lipid leaflets led to another interesting observation described in the next section of this article.

Figure 1. Superimposition of the amphiphilic region of E2 (segment 689 to 703) of three molecular dynamics simulations. The most stable helical region of the E2 amphiphilic segment during molecular dynamics simulations. 2KZQ.pdb in red, I-Tasser model in black and ideal helix model in gray.
Figure 3. (a) Molecular dynamics simulation of the ideal helix model. The amphiphilic segment was positioned in water-lipid interface and the TM domain was oriented in hydrophobic core of the bilayer. The length of the helix of residues 689–703 is plotted in red, root mean square deviation of the same residues with respect to the starting structure is plotted in black. (b) Molecular dynamics simulation of nuclear magnetic resonance derived structure (2KZQ.pdb). 2KZQ was positioned in the water-lipid interface. The length of the helix segment is plotted in red, root mean square deviation of the same residue with respect to starting structure is plotted in black.

Figure 4. Distance between the helix and lipid leaflets during molecular dynamic simulation with the I-Tasser model. The distance to the upper lipid leaflet is plotted in red and lower leaflet is plotted in black.

Figure 5. Distance of the amphiphilic segment to the lipid leaflets. The 2KZQ structure is colored in black while the ideal helix model is colored in red. Amphiphilic residues remain within 4 Å from phosphate head group throughout the simulations.

Author contributions
SAJ analyzed the sequences. RA performed the molecular dynamics simulations. SAJ and RA carried out the research, were involved in the drafting of manuscript and have approved the final content.

Competing interests
No competing interests were disclosed.

Grant information
This work was supported by the Universiti Teknologi MARA (UiTM) Dana Cluster 600-RMI/DANA 53/3/CG (2/2012). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgments
We are grateful to Faculty of Pharmacy, Universiti Teknologi MARA (UiTM) for providing the computational facilities in the Bioinformatics Lab. We acknowledge financial and administrative support from the Research Management Institute (RMI), UiTM and Ministry of Science and Technology Malaysia (MOSTI).
Supplementary figure

Supplementary Figure 1. Molecular dynamics simulation of I-Tasser model. (a) Initial structure; (b) 100 ns snapshot. (Red – residues 689–703).

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Reviewer Report 18 April 2013

https://doi.org/10.5256/f1000research.909.r888

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General Comments and Recommendation: The title is appropriate, the design of the simulations, and the methods and analysis employed are state of the art. The data provided is sufficient. However, the conclusions are overstretched and much longer simulations are needed to substantiate the claims.

More detailed review: The article describes simulation studies of a 64-residue transmembrane fragment of the envelope glycoprotein E2 domain consisting of two segments: TM (residues 715-746) and ST (residues 683-714). The objective of the study is mainly to understand the role and structural dynamics of the ST segment. MD simulations have been performed on three different starting points: 2 models and one experimentally derived structure. The experimental structure 2KZQ is only 36 residues long itself, lacking the TM segment, and has large dynamics within the NMR structural ensemble (36 models with mean RMSD of 11.32). It is the homolog that produced the best model in I-Tasser, the authors have used in the study. The authors do not mention which model from the NMR ensemble they used in their MD simulations. Though the authors refer to previous experimental studies that show that the ST segment is helical upon membrane binding, a simple sequence-based helical propensity prediction of the HCV E2 genotype 1a (H77 strain) sequence (Uniprot ID P27958) could be included (to further substantiate their 'ideal helix' model used for the MD simulations). One of the main results of the article is that the ST segment has a stable helical conformation, stabilized more by the lipid-peptide interactions than the TM-ST peptide-peptide interactions. The similarity between the three different simulations, even with one of them lacking the TM segment, forms the basis of their conclusion. The authors observe uncharacteristic spikes towards the end in the simulations of NMR-structure and the I-Tasser model MD simulations (Figures 2, 3a, 3b). Since the NMR-structure simulation is very short (just 20ns) compared to the I-Tasser model simulation, the authors could perform a longer, equivalent 100ns simulation to compare them and ascertain further the importance of lipid-peptide
interactions in the ST helical segment stability. Also, given that in the I-Tasser simulation, there is a lot of dynamics observed after the 20ns timepoint until the end of the simulation, a longer simulation of the NMR-structure is recommended. The other key result which we found interesting is the orientational and positional preference of the amphipathic ST segment, which seems to be parallel to the lipid leaflets. Reorganization and movement of the ST segment towards the hydrophobic core of the lipids, as shown in Fig S1, is observed clearly in the I-Tasser model simulation, whereas the initial horizontal orientation is maintained in the other simulations. The authors could identify the key residues involved in this re-organization and highlight them

**Competing Interests:** No competing interests were disclosed.

**We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.**

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**Reviewer Report 18 April 2013**

https://doi.org/10.5256/f1000research.909.r900

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**Gloria Fuentes**

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The authors have carried out MD simulations in lipid bilayer for three different models for the C-terminal region of the envelope glycoprotein E2. The use of different structures provides *a priori* a good benchmarking for the observations claimed in the article. However, the trajectories presented in this work seem not to have converged completely. The plots will show the trend more clearly if running average is used rather than the raw data. With the current advances in hardware and software, it seems to me that the authors have explored a limited conformational space of the system, which might be masking some other biological conformations for the region in the study.

I am missing a more elaborated discussion in context with the available experimental data that they refer to in the conclusion. So far the correlation done in this study is too vague. The paper with experimental data the authors mentioned also suggests that this amphipathic helix could fold upon lipid binding. A MD simulation in water could help to elucidate this experimental observation. It would be convenient if the authors could specify the exact definition of membrane interface; for those people not working in the field, it could be a bit confusing. This concept could suggest the interface of the phospholipid bilayer (membrane core) or the lipid-water interface.

The legend for the Supplementary Figure is missing the colour code used.

**Competing Interests:** No competing interests were disclosed.
The paper describes comparative MD simulations on the E2 TM region embedded in a lipid membrane. Different start conformations have been considered based on different molecular modelling methods + a structure from the pdb. I believe the simulations have been performed properly. Some interesting results on orientational preference of the helices have been reported. However, it should be emphasized that the simulations are too short to achieve convergence of the simulation results. This should be discussed in the Results & Discussion section or in the Conclusion section. Also, comparison to other simulation studies on peptide helices in membranes are missing (e.g. of the Thielemann group). At the end of the conclusion section the authors state “The results also revealed the orientation and positional preferences of the amphiphilic segment in relation to the water-bilayer interface that further clarify speculations from experimental studies.”

The authors mention “.. speculations from experimental studies.”, however, no reference to such speculations have been given and the details of the speculations are not discussed. The authors should give proper reference and should in detail explain what is meant by their statement.

**Competing Interests:** No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.
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