Exploring the Membrane Fusion Mechanism through Force-Induced Disassembly of HIV-1 Six-Helix Bundle

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Abstract

Enveloped virus, such as HIV-1, employs membrane fusion mechanism to invade into host cell. HIV-1 gp41 ectodomain uses six-helix bundle configuration to accomplish this process. Using molecular dynamic simulations, we confirmed the stability of this six-helix bundle by showing high occupancy of hydrogen bonds and hydrophobic interactions. Key residues and interactions important for the bundle integration were characterized by force-induced unfolding simulations of six-helix bundle, exhibiting the collapse order of these groups of interactions. Moreover, our results in some way concerted with a previous theory that the formation of coiled-coil choose a route which involved cooperative interactions between the N-terminal and C-terminal helix.

Abbreviations

MD, molecular dynamics; SMD, steered molecular dynamics; 6HB, six-helix bundle; NHR/CHR, N/C-terminal heptad repeat; RMSD, root-mean-square deviation; RMSF, root-mean-square fluctuation.

Keywords

Six-helix bundle; Protein unfolding; Molecular dynamics simulations; Interfacial interaction; Membrane fusion.

1. Introduction

HIV-1 envelope, like many other viral fusion proteins such as Influenza hemagglutinin and Ebola envelope, uses a six-helix bundle (6HB) configuration as driving force for membrane fusion[1]. HIV-1 envelope glycoprotein (Env) spikes, containing three gp120 and three gp41 subunits, use the trimeric conformation to fuse into host cells and initiate infection. Through decades of investigation[2,3,4,5,6], it has been well though not enough understood how envelope proteins take shape and functions.

HIV-1 membrane fusion occurs at the interaction of gp120 components with CD4 receptors and CCR5 or CXCR4 co-receptors sequentially, consequently changes the association of gp120 and gp41 then induces conformational change of gp41. The ectodomain of gp41 extends itself out from the viral membrane, three of which form a coiled-coil configuration. Previous study [7] shows that each HIV-1 gp41 ectodomain contains two heptad-repeat helices (N-terminal and C-terminal heptad repeats, NHR & CHR) arranged anti-parallel, each of which is split into smaller helices connected by loops in pre-fusion state. Before membrane fusion commences, three gp120 subunits act as a 'safety pin' to prevent gp41 from transiting to 6HB hairpin conformation which should be more energetically favored. A better understanding of membrane fusion process is inevitably important for the further development of immunologic and pharmacologic strategies in preventing HIV-1 infection. Mutagenesis studies have been indicating that some bulky hydrophobic residues contribute to the stability of gp41 6HB and the inhibitory feature of CHR, suggesting that interfacial interactions inside the fusogenic hairpin structure might serve as the basis for antiviral drugs [8,9,10,11,12,13].

Molecular dynamics (MD) simulation is a useful tool for understanding the structure and function of biological macromolecules, providing remarkable insights into various kinds of proteins, nuclear acids, membranes and their complexes[14,15]. Based on the crystal structure of HIV-1 gp41 ectodomain, we build a model for the post-fusion configuration of HIV-1 gp41 in a solvent environment and examine the performance of gp41 6HB unfolding using different pulling strategies adopting steered molecular dynamics (SMD). Our results demonstrate that HIV-1 post-fusion structure maintains a continuous helix, which adopts an extremely stable 6HB configuration due to the high occupancy of hydrogen bonds as well as hydrophobic

interactions. Additionally, the effects of some key residues are characterized during the transition from 6HB to gp41 intermediate state. And our result further corroborates a previous theory that class I viral fusion proteins, might resort to a cooperative route in which after one fusion peptide inserts into the viral membrane and the formation of one C-terminal helix aids in the remaining into the host membrane and thus forms the final 6HB configuration[16].

2. Materials & Methods

2.1. *System preparation and equilibration*

The crystal structure of the ectodomain of HIV-1 gp41 (PDB code 1ENV) was used as the initial coordinates of the 6HB for the MD simulations[3].

The Q540E mutation introduced in the original PDB structure was changed back to the wide-type sequence using the mutagenesis tool of VMD. The protonation states of the histidine residues were identified by PDB2PQR Server 2.0 [17] and the hydrogen atoms were added assuming pH 7.0. Solvation using TIP3P water [18] was performed with a 150 Å padding in the negative z axis direction and 13 Å padding in the other directions, resulting a rectangular water box (approximately 31,000 water molecules). Then Na^+ and Cl^- counterions were added to neutralize the system and maintain ion concentration of 0.15 M. The final system for MD simulations contains 97,826 atoms (Supporting Fig. S1).

All-atom MD and SMD simulations were performed using the package NAMD 2.9 [19]. The CHARMM27 force field with CMAP corrections [20,21] was used for all simulations. All free MD simulations employed Langevin-based temperature control at 310 K with a coupling constant of 1ps^{-1} . Long-range electrostatics were handled using particle-mesh Ewald summation with a grid spacing of 1 Å. Non-bonded interactions were cut off at 12 Å. The system was initially energy-minimized for 10,000steps and equilibrated for 3 ns employing a time step of 1 fs, including 1 ns with all heavy atoms harmonically constrained and 1 ns with protein backbone harmonically constrained under NVT ensemble followed by 1 ns under NPT ensemble. Afterwards, an equilibration of 100 ns was performed under NPT ensemble with a time step of 2 fs.

2.2. *Steered molecular dynamics*

Starting from the 50ns and 100ns snapshots of equilibration period, we performed subsequent two rounds of 2ns simulations under NPT and NV ensembles with the time step of 1 fs and the resulted configurations were used for steered molecular dynamics (SMD) simulations. Two sets of SMD simulations, including ten individuals (Sim. S1-S10), are carried out, as listed in Table S1. In the first sets of SMD simulations (Sim. S1-S6), depicted in Fig.2A, C α of N-terminal Q540 of helix AN was fixed and C α atom of C-terminal D664 of helices AC, BC, or CC was pulled by a spring which moved at constant speed, respectively. In the second sets of SMD simulations (Sim. S7-S10), depicted in Fig.2A, C α atom of residue Q540 of all three chains were fixed and force was applied to all three C α atoms of residue D664 at the same time. For Sim. S7 and S8, pulling speed $v = 0.2$ nm/ns was used, and the spring constant was chosen as $k = 0.1$ kcal/mol/Å² (~ 70 pN/nm), while for Sim. S9 and S10, those three CHRs were each pulled at a constant force of 400 pN, respectively. Pulling in the negative z direction was used for all SMD runs, in which approximately 90 ns was needed to entirely dissociate the helix from 6HB.

Geometry analysis, molecular images and movies in this study were rendered using the molecular visualization software VMD 1.9.2 [22].

3. Results & Discussion

3.1. *MD simulations confirm the stability of the gp41 6HB configuration*

The transition of HIV-1 6HB accomplishes the last phase of membrane fusion[23]. 6HB is formed by three copies of NHRs and CHRs linked by disulfide loop which was not solved in the crystal structure. In our simulations, we used a truncated form of 6HB to reduce the system size (Fig. 1A-B).

The stability of the 6HB was verified by a 100 ns free MD simulation. Root-mean-square deviations (RMSD) of the backbone atoms with respect to the starting coordinates over the simulation is plotted (Fig. 1C), which reached its plateau soon after the simulation started, indicating that the 6HB conformations had reach and stayed in equilibrium. The per-residue root-mean-square fluctuations (RMSFs) of C α atoms were computed (Fig. 1D & Fig. S2) to

track the extent of variation of the individual residues. The results indicate that all residues underwent small fluctuation except for those at the ends. These results are consistent with the fact that 6HB is stable and the final configuration of HIV-1 envelope protein's ectodomain during the whole membrane fusion process. Geometry analysis presents that there exist fifteen to twenty hydrogen bonds and two main hydrophobic regions among these six helices (Fig. S3).

Our simulations demonstrate that HIV-1 post-fusion structure maintains a continuous helix, which adopts an extremely stable 6HB configuration due to the high occupancy of hydrogen bonds as well as hydrophobic interaction. The stability of gp41 6HB suggests that this complex is located at a low-energy conformation and large conformation change hardly happens to this 6HB complex without any external effect.

3.2. Interfacial interactions inside 6HB contribute to the stability of HIV-1 envelope post-fusion conformation

Force-driven unfolding of single helix from 6HB (Sim. S1-S6) is used to address the key residues or interactions that work in the integration of 6HB, which is essential to promote the membrane fusion. Pulling each segment individually can provide us information on the packing strength of the segment to the fixed segment, also it is helpful to compare the simulation results with recent single-molecule force experiments with optical tweezers [24].

Considering that the coupling of NHRs and CHR is the key step of membrane fusion and the three NHRs(CHR)s helices are almost symmetric, the N-terminal of one of NHR helix is fixed and the C-terminal of one of CHR helix is pulled. Although their force-time curves differed significantly (data not shown), force-extension curves of these six SMD simulations all showed five distinct peaks even in different pulling strategies (Fig. 4B), in which some different features can still be found. By fixing the N-terminal of helix AN, Sim S1, S2, S5 and S6, pulling the C-terminal of helix AC or CC showed the similar F-E curves, which is different with that of Sim S3, S4 (pulling the C-terminal of BC helix). It might result from the fact that AN helix interacts directly with the helices AC and CC, but BC does not interact with AN directly.

Five groups of interfacial interactions composed of the hydrophobic interactions or hydrogen bonds were characterized and corresponded to the peaks of force-extension curves as the indicated order (Fig.2B-C). These interactions existed at interfaces between helices

AN-AC-CN, BN-BC-AN or CN-CC-BN (Table. 1), but only those between helices AN-AC-CN were exemplified (Fig. 2C). During SMD simulations, all C-terminal C α atoms started to deviate from the fixed N-terminal C α atom from their equilibrium positions. This onset coincided with the first two force peaks (① and ②) involving L663, L660, L554 and V549. Subsequently, hydrogen bonds between S649/Q653 and Q551 (③) began to break, and the change of distance contributed to the fact that polar interaction between Q653-Q551/S649-Q551 was responsible for the conformational change of 6HB, while they also had quite a large hydrogen bonding occupancy throughout the equilibrium (Fig. 3 & Table. 2). This interaction between side chains of S649 and Q551 was proposed in a previous investigation of HIV-1 6HB [25]. While S640 was mutated to alanine or aspartic acid, along with the I559P, V570D and I573D mutations, 6HB formation could be destabilized[10]. Lastly, as the last two groups of hydrophobic interactions among I635, W631, W628, I573, W571, V570, L568 and L548 (④ and ⑤) crashed, the whole CHR dissociated with the complex. Previous study shows residues constituting 6HB's highly conserved, hydrophobic cavity is composed of L566, V570, K574, Q577 of one NHR, L565, L568, W571, G572 of another NHR and T569, I573, L576 from both NHRs, which accommodates three hydrophobic residues (I635, W631, W628) from the abutting CHR, stabilizing the structure of 6HB collectively[9]. Yet, previously demonstrated spatial distance contributed to the different force profiles in Sim S3 and S4. For instance, in the case of S3 and S4, the force needed to break interactions between residue L544 on helix AN and L660/L663 on helix BC was much smaller because there exists constraint on helix AN, leading to a bigger energy barrier. While in S1, S2, S5 and S6, this group of interactions sat between helices CN/AC or CC/BN in which there was not any constraints. It is found that these five interaction patterns contain four groups of hydrophobic interaction and one group of polar interaction, which suggests that hydrophobic interaction perhaps is more important for the integration of gp41 6HB. More detailed structural changes along the trajectory are illustrated in Fig. S4 and Movies S1, S2 and S3.

The results of this set of simulations are in agree with a recent experimental study with optical tweezers [24]. While in our simulations, only the dissociation of one helix from the rest part of the bundle is studied, and we have identified more intermediate states as discussed above due to the atomistic resolution of the MD simulations.

3.3. *The coiled-coil 6HB formation might conduct a cooperative way*

The detailed molecular process of 6HB formation is not fully understood. A key question was the relationship among the three CHR_s. To gain insight on this issue, we performed another sets of simulations where all the CHR_s were pulled at the same time.

The external force was applied to three C-terminal C α atoms to observe the response of 6HB (Fig.4A), which is more closely related with function state during the membrane function. Both trajectories (Sim S7, S8) showed the similar force profiles with each other although starting from two different conformations (Fig. 4B). However, compared to the single helix unfolding (Sim S1-S6), Sim. S7 and S8 behaved very distinctly. The gp41 6HB hold its overall structure well in the first 60ns, and the applied force increases almost linearly. As the force is large enough, one or two CHR_s dissociated from the 6HB complex instantly, and the other NHR_s and CHR_s still keep coiled-coil conformation (Fig. 4C& Fig. S4). This behavior with no doubts has something to do with the fact that there were three NHR_s internally arranged, causing steric hindrance between three CHR_s. It is interesting that asymmetric unfolding of three CHR_s take place in spite of the approximate symmetric structure of gp41 6HB. We believe that this should be related to the gp41 6HB formation, and the membrane fusion. A previously proposed theory described two energetically accessible routes, which class I viral fusion proteins, including Influenza hemagglutinin, HIV-1 and Ebola envelope adopt, to complete the coiled-coil transition: a sequential route and a cooperative route. The latter suggested that the formation of coiled-coil involves cooperative interactions between the N-terminal and C-terminal helix [16]. However, in these pulling simulations, the force the other chain experienced will increase rapidly after one of those CHR_s was separated from the bundle, which may provide an alternative explanation on the results other than cooperative binding. To further confirm the cooperativity, we performed new sets of simulations (Sim S9, S10), in which the C-terminals of the three CHR_s were pulled separately with constant forces, each ~400 pN. From the results (Fig. 4D), it is obvious that all three CHR_s start to dissociate from the NHR_s at about the same time, which strongly indicated that the three CHR_s bound with the NHR_s cooperatively.

Acknowledgments

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Tables

Table 1. Five groups of interfacial interactions identified in S1-S2 simulations.

No. *	Residues at [#]			Interaction Type
	Helix AN	Helix AC	Helix CN	
①		L663/L660	L554	Hydrophobic
②	V549	L660	L554	Hydrophobic
③	--	S649/Q653	Q551	Hydrogen Bonds
④	V570	I635	L548	Hydrophobic
⑤	I573/V570	W631/W628	W571/L568	Hydrophobic

*Numbers designated in this table correspond to the five peaks in Fig. 2B.

The same interactions exist in the simulations of S3-S4 (BN-BC-AN) or S5-S6 (CN-CC-BN).

Table 2. Hydrogen bonding pairs in equilibration.

Residue Pair	Helix Pair	Occupancy*
	AC-CN	96.60%
Q653-Q551	BC-AN	99.00%
	CC-BN	96.60%
	AC-CN	12.70%
S649-Q551	BC-AN	23.60%
	CC-BN	16.60%

*Hydrogen bond is defined as that the distance between two polar heavy atoms is less than 3.5 Å.

Figures

Fig. 1. (A) Crystal structure of a gp41 6HB formed by HIV-1 N41 & C37. The left panel shows a lateral view of 6HB. The right panel shows an axial view looking down the 3-fold axis of 6HB. Helices of different chains are indicated. (B) Schematic view of the HIV-1 gp41 ectodomain. Two hydrophobic NHR and CHR are indicated. The model consists of 3 repeats of N41 and 3 repeats of C37 peptide. The sequence of the HIV-1 gp41 model is shown. The residues are numbered according to their position in gp160 of the HXB2 HIV-1 strain. (C) RMSDs for backbone atoms from the original gp41 6HB throughout the equilibrium. (D) RMSFs for gp41 6HB backbone C α atoms mapped to ribbon diagram for the three subunits. High to low RMSFs are colored from red, white to blue.

Fig. 2. Force-driven unfolding of single helix in gp41 6HB. (A) Set-up of S1-S6 simulations. Arrows show the direction of applied force in these six simulations. (B) Force-Extension curves in S1-S6 simulations, in which the extension is defined as the distance between pulling atoms and fixed atoms in the direction of applied force. (C) Interaction partners between NHR and CHR responsible for the force peak indicated in Force-Extension curves, where ①, ②, ④ and ⑤ is the hydrophobic interaction and ③ is the polar interaction.

Fig. 3. Key polar interaction residues identified by SMDs: Q653-Q551 & S649-Q551. The distances in the residue pair Q653-Q551 (Left) & S649-Q551 (Right) for Sim. S1-S6 are monitored.

Fig. 4. Force-driven unfolding of gp41 6HB with 3 N-terminal α fixed. (A) Set-up of S7/S8 simulations. (B) Force-Extension curves of SMD trajectories. The position change curve of three pulled atoms at constant velocity when they are pulled together (C) or constant force when they are pulled separately (D). The coloring scheme is the same as in A.