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Correlation of biological activity with computationally derived structural features from transmembrane hetero-dimers of HIV-1 Vpu with host factors ☆

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ABSTRACT

Vpu is an 81 amino acid type I integral membrane protein encoded by human immunodeficiency virus type 1 (HIV-1). It is identified to support viral release by potentially forming ion and substrate conducting channels and by modulating the function of host factors. The focus is on the interaction of the transmembrane domains of Vpu with those of host factors using a combination of molecular dynamics simulations and docking approach. Binding poses and adopted tilt angles of the dimers are analyzed and correlated with experimentally derived activity data from literature. Vpu activity is driven by dimerization with the host protein *via* its alanine rim Ala-8/11/15/19. Tight binding is shown by an almost parallel alignment of the helices in the dimers. Less parallel alignment is proposed to correlate with lower activity. This article is part of a Special Issue entitled: Viral Membrane Proteins — Channels for Cellular Networking.

1. Introduction

Viral channel proteins belong to the class of proteins that self-assemble into homo-oligomers enabling ion and substrate flux across the lipid membrane [1,2]. The consequence of this is an altered environment for improved reproduction of the virus. It has been shown that the mode of action of these proteins is important in the early stage of viral entry into the host, e.g. for Kcv from PBCV-1 [3], and in the later stage of the infectivity cycle, e.g. for Vpu from HIV-1 (reviewed in [4]). In the case of the viral proton conducting channel M2 from influenza A, the protein is found to be essential during the early stage as well as during the later stage, while other viral proteins need to be manufactured [5,6]. In all the stages mentioned it is rather the proton/ion flux which is thought to trigger downstream molecular mechanical events.

In recent years it has become evident that Vpu from HIV-1 is interacting with a number of host factors such as CD4, BST-2 and NTB-A (reviewed in [1]). Similarly, E5 of human papillomavirus 16 has recently been identified to release ions and substrates using fluorescent dye release essays with liposomes [7]. E5 is declared as a member of the channel forming viral proteins and is also known for its interaction with a series of host factors [8,9]. This sparks thoughts about the importance of the role of channel formation in comparison to the role of manipulating the host cell via interaction with host-factors (for Vpu see [10,11]). How these proteins 'find' their partners, and how strong the interaction to the attached host proteins must be in order to be processed further are still to be elucidated.

Vpu is an 81 amino acid type I integral membrane protein found in HIV-1 [12,13] and related chimpanzee isolate SIV_{CPZ} [14]. As an auxiliary protein it amplifies virus replication [15] (for review see [16–18]). Shortly after its discovery the mechanism of amplification has been attributed to its capability to oligomerize [19–21], to form channels within the lipid membrane [22] and to down-regulate the receptor protein CD4 [23]. In the first case channel formation was found to be solely due to the transmembrane domain (TMD) of Vpu. Randomization of the TMD of Vpu leads to an abrogation of channel activity [22]. In the second case the cytoplasmic domain was identified to be essential. In recent years more host factors have been identified such as CD317/tetherin/BST-2 [24–26], CD74 [27] and NTB-A [28] with which Vpu is supposed to interact. The consequence of the interaction is that these proteins are redirected to the ubiquitin-dependent proteasome degradation pathway. In the case of both CD317/tetherin/BST-2 [24–26,29] and NTB-A [28] the TMD of each of the membrane proteins is the target of Vpu.

NMR spectroscopic experiments of peptides corresponding to the TMD of Vpu reveal a helix which is kinked around Ile-17 [30–33]. The kink is also found in molecular dynamics (MD) simulations of the TMD when embedded into hydrated lipid bilayers and includes Ile-20 to Ser-24 [34]. The physical interaction of the TMDs of Vpu and BST-2 is confirmed by NMR spectroscopy to be driven by hydrophobic interactions between the two helices [35]. The alanine rim of Vpu allows larger residues within the TMD of BST-2, such as V-30, I-34 and L-37, to

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interact tightly. The alanine rim follows an AxxxAxxxA motif which is conserved among Vpu isolates [36]. This motif adds up to a series of known motifs for TMD–TMD interactions [37,38].

Computational protocols are proposed to generate dimeric and oligomeric assemblies of membrane proteins and peptides based on the TMDs using docking based screening of the interhelical interactions [39–43], (reviewed in [44]). There have been attempts to derive TMD-oligomerization by allowing full scale protein dynamics by simulating the process in a lipid bilayer environment [40,45,46].

In this study we focus on the structural features and dynamics of the TMDs of Vpu and Vpu mutants once assembled into dimers either with BST-2, CD4 or NTB-A. In each case, the TMDs are known to be the segments of the proteins relevant for their biological function. While BST-2, NTB-A and Vpu interact *via* their TMDs, for Vpu and CD4 they seem not to be the only contact point. It is investigated whether results from MD simulations can be correlated with related biological experiments. Vpu is not necessarily co-down-regulated with all host factors used in this study. It is anticipated that there has to be a structural and dynamical aspect in the interaction because Vpu needs obviously also to dissociate from the host-TMD, especially while interacting e.g. with BST-2.

This study is seen as an attempt to use the tools in a computational bioanalytical approach. The helical motif of TMDs can easily be modeled based on bioinformatics tools such as secondary structure prediction programs. Limiting the study to the TMDs is, besides the biological relevance of these domains as mentioned, also due to minimize calculation time when screening larger data sets.

2. Materials and methods

Ideal helices ($\phi = -65°$, $\psi = -39°$) of the N terminal side of Vpu (Vpu HV1H2), including the TMD of the protein as well as the TMDs of CD4, BST-2 and NTB-A were generated using the program MOE2008.10 (Molecular Operation Environment, www.chemcomp.com):

Vpu_{1-32} (Vpu)	MQPIPIVAIV[10] ALVVAIIIAI[20] VVWSIVIIEY[30] RK
Random Vpu_{1-27} (RVpu)	MIPIVIAIIL[10] AVAVQAIVIV[20] IVSWIIE
$CD4_{397-418}$ (CD4)	MALI[400] VLGGVAGLLL[410] FIGLGIFF
$BST\text{-}2_{5-27}$ (BST-2)	LLGIGI[10] LVLLIVILG[20] VPLIIF
$NTB\text{-}A_{229-249}$ (NTB-A)	FM[230] VSGICIVFGF[240] IILLLLVLA.

The sequence of RVpu was taken from [22]. The mutations of the TMD of Vpu_{1-32} are done as following A19H, A19L, A19N, A19F, A15L, A15N, A15F, W23A, I16/17/18T (I3xT), and I16/17/18V (I3xV). This four letter code is used in the text.

2.1. Preparation of the protein/lipid/water system

Proteins, uncharged at both ends, were embedded into POPC lipid bilayer patches (POPC: 16:0–18:1 diester PC, 1-palmitoyl-2-oleoyl-*sn*glycero-3-phosphocholine). Prior to protein insertion, patches of 128 lipids were equilibrated for 70 ns [34] and used for the simulations.

Individual helices and protein assemblies were inserted into the POPC bilayer patches using the MOE software package. Lipids were manually removed to avoid an overlapping with the proteins. Finally, the patches consisted of 122 lipids (6344 atoms). The protein/lipid system was hydrated with about 3655 water molecules.

All MD simulations were carried out using GROMACS 4.0.5 with the Gromos96 (ffG45a3) force field. Peptides, lipids, and the water molecules were separately coupled to a Berendsen thermostat at 310 K with a coupling time of 0.1 ps. The compressibility was set to 4.5×10^{-5} bar^{-1}. The monomers were simulated using a semi-isotropic pressure coupling scheme. Long range electrostatics was calculated using the particle-mesh Ewald (PME) algorithm with grid dimensions of 0.12 nm and interpolation order 4. Lennard–Jones and short-range Coulomb interactions were cut off at 1.4 and 0.9 nm, respectively. Water molecules were represented by the SPC model. The protein/lipid/water system was energy minimized (1000 steps steepest descents, 5000 steps of conjugate gradient) followed by a total of 1.9 ns (122 lipid patch) of equilibration MD simulation. The following equilibration protocol was used: (i) the temperature was gradually increased from 100 K to 200 K and 310 K. The system was run for 200 ps for the first two temperatures and 1.5 ns for the latter (500 ps for the patch containing 122 lipids). During these simulations the protein remained fully restraint ($k = 1000$ kJ mol^{-1}). At 310 K the restraints kept on the protein *via* the force constant k, were released in 2 steps from $k = 500$ kJ mol^{-1} nm^{-2} to $k = 250$ kJ mol^{-1} nm^{-2}. Each step was run for 1.5 ns. For the system containing 122 lipids, 2 steps ($k = 500$ kJ mol^{-1} nm^{-2} and $k = 250$ kJ mol^{-1} nm^{-2}) were used each running for 500 ps.

2.2. Assembly

The starting structure for the assembly of TMDs was the average structure over the backbone atoms of the 100 ns MD simulations. Rotational and translational motions were removed by fitting the peptide structure of each time frame to the starting structure. The program g_covar from the GROMACS-3.3.1 and 4.0.5 packages was used for the calculations [42].

The dimers were assembled using a program based on the scripting language 'scientific vector language' (SLV) of the MOE suit as reported earlier [42,43]. For energy calculations the AMBER 94 force field was used. To simulate the assembly within the lipid bilayer the dielectric constant (ε) was set as $\varepsilon = 2$. The helical backbone structures were aligned along the *z*-axis. The conformational space of the assembly was screened by keeping one helix fixed but free to rotate around its own axis while the second helix was able to move in respect to the interhelical distance, its tilt in respect to the other helix and to rotate as well around its own axis. All dimers were generated by screening the interhelical distance in steps of 0.25 Å, the tilt and rotational angles in steps of 2° and 5°, respectively. Interhelical distances were varied between 8 and 13 Å for each peptide, and the tilt was varied between ±36°. The assembly protocol usually generated about 350,000 conformers which were stored in a data base for further analysis.

Plots of the root mean square fluctuation (RMSF) of the Cα atoms of each residue as well as calculations of the tilt and kink angles were generated over the last 70 ns of the 100 ns simulation and over the entire duration of the 200 ns simulation run. The tilts and kink are measured using the coordinates of the center of mass calculated from the backbone of residues 5–8, 20–23 and 24–28.

The simulations were prepared on a DELL T7500 workstation and submitted to the National Center for High Performance Computing (NCHC), Hsinchu, TW using 24 cpu's in parallel for 168 h for the production run of 200 ns.

Plots and pictures were made with VMD-1.8.7, Origin 8.5 and Pymol.

3. Results

The helical motif of each of the TMDs of Vpu, CD4, BST-2, NTB-A, A19H, I3xT and W23A remains intact during a 100 ns MD simulation (Fig. 1A). The RMSD shows the general pattern of a rise which is plateauing off after the first ns (Fig. 1B). The RMSF values express a *w*-like shape for all the TMDs (Fig. 1C). Mutant A19H shows the highest dynamics around Ile-16 and the mutated His-19 (Fig. 1C, II) compared with the other peptides. Residues from the middle of the helix towards the C terminus exhibit a gradual increase in fluctuation for BST-2. The respective values for any mutant Vpu_{1-32} show the same trend for both RMSD and RMSF values as shown in Fig. 1 (data not shown).

While assembling the TMDs mentioned above and those of other mutant of Vpu_{1-32} with BST-2 using the docking software, the distance between the dimers ranges between 9.5 and 12.75 Å (Table 1, Suppl.