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Qualitative computational bioanalytics: Assembly of viral channel-forming peptides around mono and divalent ions

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ABSTRACT

A fine-grained docking protocol was used to generate a bundle-like structure of the bitopic membrane protein Vpu from HIV-1. Vpu is a type I membrane protein with 81 amino acids. It is proposed that Vpu forms ion- and substrate-conducting bundles, which are located at the plasma membrane in the infected cell. The Vpu_{1-32} peptide that includes the transmembrane domain (TMD) is assembled into homo-pentameric bundles around prepositioned Na, K, Ca or Cl ions. For bundles with the lowest energy, the TMDs generate a hydrophobic pore. Bundles in which Ser-24 faces the pore have higher energy. The tilt of the helices in the lowest energy bundles is larger than bundles with serines facing the pore. Left-handed bundles are lowest in energy where the ions are located at the serines.

1. Introduction

Computation is an essential tool that supports various applications, such as the drug discovery process and material sciences. It is also an essential part of the available range of analytical tools [1]: e.g. it has been suggested by computational methods that additional potassium ions must be included in an ion channel structure [2]; independent from this suggestion, potassium ions have been observed in experiments [3]. This underlines the importance of applying computational methods to structure prediction. Computational methods are indispensable for deciphering patterns of protein–protein interaction, especially for transmembrane proteins [4]. In this study, a computational bioanalytical approach is applied to investigate the architecture of bundles formed by the channel-forming membrane protein Vpu from human immunodeficiency virus type 1 (HIV-1) (reviewed in [5]) in the presence of mono and divalent ions.

Vpu is a type I integral membrane protein with 81 amino acids [6,7]. It is expressed during the late stage of the infectivity cycle and enhances viral release [6,8]. This activity is attributed to the interaction between Vpu and the host factor CD317/BST-2/tetherin [9,10]. When Vpu and BST-2 interact, BST-2 is susceptible to ubiquitin-dependent down-regulation (reviewed in [11,12]). The Vpu TMD is the interaction site for BST-2 [9,10]. Before the Vpu and BST-2 interaction was discovered, viral release enhancement was solely correlated with the Vpu capacity for channel formation at the plasma membrane [13,14]. Vpu and its TMD has been reconstituted into an artificial lipid bilayer, which renderes the lipid membrane permeable to physiologically relevant ions [14–16] and small molecules [17]. The channel is also active when Vpu was expressed in *Xenopus* oocytes [14] or 293T cells [18], which is measured using a whole-cell recording experiment. However, this function has not been verified within the life cycle of the virus.

Structurally, Vpu has been reasonably well-explored by a series of spectroscopic measurements and computer simulations (reviewed in [5]). Emphasising the latter, simulations of assembled Vpu bundle TMDs have been performed primarily for a pentameric bundle [19–22]. The only hydrophilic residue, Ser-24, faces the bundle lumen in these assemblies. Only one study suggests that Trp-23 points towards the lumen [23]. Tetrameric and pentameric bundles were modelled using NMR spectroscopic data [24]. In these bundles, residues Trp-23 and Ser-24 did not face the inside of the bundles, but the hydrophobic residues form a 'hydrophobic' pore.

Results from a fine-grained docking approach with molecular dynamics simulations show that the lowest energy structures for the trimeric, tetrameric and pentameric Vpu bundles comprise the hydrophobic residues inside the lumen [25]. Structures with the serines facing the pore and forming a bundle with a 'hydrophilic' pore have high energy. These computational assembly experiments are performed without ions.

As mentioned above, investigations on KcsA have sparked the idea that the Vpu bundle should be assembled in the presence of

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ions. In such a protocol, the serines are expected to face the pore lumen in the lowest energy structures.

Calculations are performed for Vpu and the pore-lining M2 structural motif of proton-activated pentameric ligand-gated ion channel from *Gloebacter violaceus* (GLIC) representing an open channel [26]. Herein, helices, including the Vpu TMD, are assembled in the presence of ions using an established fine-grained docking approach [27].

2. Materials and methods

Ideal helices ($\phi = -65°$, $\psi = -39°$) in the Vpu N-terminus (Vpu HV1H2), including the TMD, were generated using the program MOE2008.10 (Molecular Operation Environment, www.chemcomp.com).

Vpu_{1-32}: MQPIPIVAIV[10] ALVVAIIIAI[20] VVWSIVIIEY[30] RK

The coordinates for the following GLIC sequence [26] are from the PDB data bank entry 3EHZ and were used without further modification:

M2: SY[220] EANVTLVVST[230] LIAHIAFNIL[240] VETN.

Both ends were modelled as uncharged groups.

2.1. MD simulations

Details on molecular dynamics (MD) simulation preparation and performance for Vpu_{1-32} have been reported elsewhere [25] and are briefly outlined herein.

Vpu_{1-32}, which is uncharged at both ends, was embedded into an equilibrated patch of a POPC lipid bilayer (POPC: 16:0–18:1 diester PC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine) with 128 lipids using the MOE software package. The lipids were manually removed to avoid overlapping with the peptide. The patches comprised 122 lipids (6344 atoms). The protein/lipid system was hydrated with 3655 water molecules. The pentameric ion containing bundles of Vpu_{1-32} were embedded in a respective lipid patch of 258 lipids (originally 288 lipids) and 8748 water molecules.

The MD simulations were performed using GROMACS 4.0.5 with the Gromos96 (ffG45a3) force field. The peptides, lipids, and water molecules were separately coupled to a Berendsen thermostat at 310 K with a 0.1 ps coupling time. Compressibility was set to 4.5e-5 bar^{-1}. The monomers were simulated with a semi-isotropic pressure coupling scheme. The long-range electrostatics was calculated using the particle-mesh Ewald (PME) algorithm with grid dimensions at 0.12 nm and the 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 using the SPC model. The protein/lipid/water system was energy-minimised followed by 1.9 ns of equilibration MD simulation. The equilibration MD simulation began with a temperature increase in the system from 100 to 200 K and to 310 K. During these simulations, the peptide was restrained. At 310 K, the peptide restraints were gradually released in three steps (k = 1000 kJ mol^{-1} nm^{-2}, k = 500 kJ mol^{-1} - nm^{-2}, k = 250 kJ mol^{-1} nm^{-2}) running each step for 1.5 ns. During a 50 ns MD simulation the ions were restraint at their position at all stages during the simulation protocol.

2.2. Assembly

An average structure for the helices' backbone residues was calculated, as reported earlier [27]. Rotational and translational movements in the helices were cancelled out by fitting each consecutive helix structure to the first structure. The structures from the last 10 ns of simulation were averaged using the program g_covar from the GROMACS-4.0.7 package.

The monomers were assembled (see also [25,27]) using a program based on the scripting 'scientific vector language' (SVL) of the MOE suit. For energy calculations, the AMBER 94 force field was used with the dielectric constant at $\varepsilon = 2$. The helices were copied, positioned around a central axis (C5 symmetry), and aligned. Each ion, K^+, Na^+, Cl^- and Ca^{2+}, was placed along the central axis at various positions. Approximately 350,000 conformers were generated by sampling interhelical distances between 10 and 15 Å then screening the tilt between ±36° and the rotational angle. Each structure was minimised.

The solvent accessible surface (SAS) was calculated using the program g_SAS of the gromacs suit.

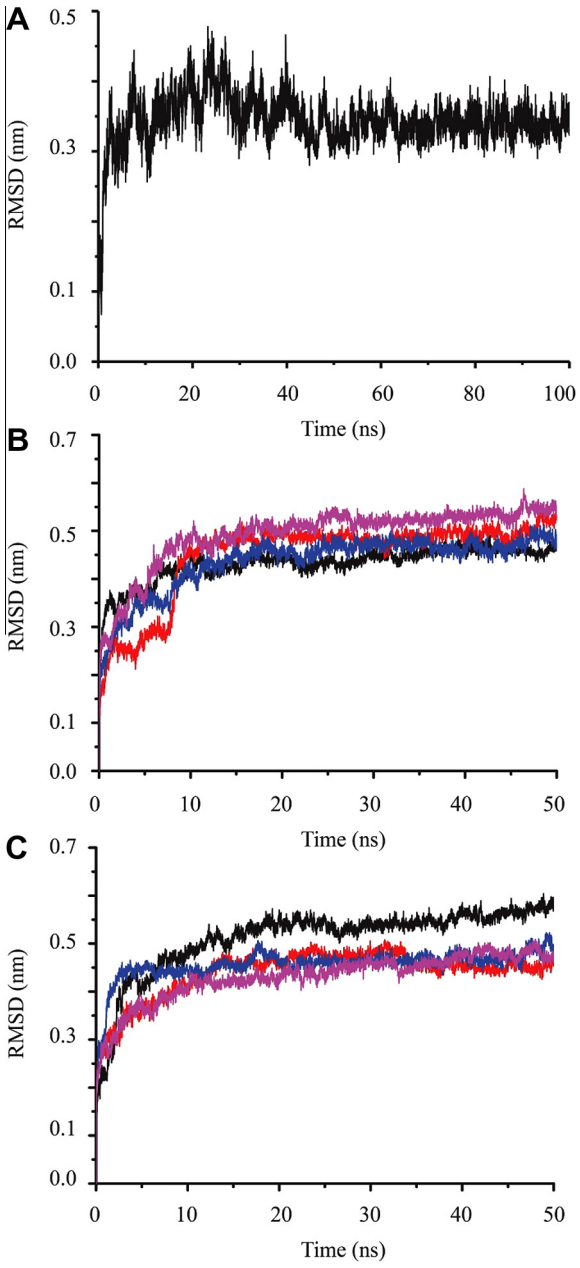

Fig. 1. RMSD values based on the Cα atoms of Vpu_{1-32} (A), the lowest-energy bundles (B) and serines-facing-pore bundles (C) of Vpu_{1-32} in the presence of restrained ions. Traces show the values for bundles with Na^+ in pink, K^+ in blue, Cl^- in red and Ca^{2+} in black. (For interpretation of color in this Figure, the reader is referred to the web version of this article.)