Simulation of the HIV-1 Vpu transmembrane domain as a pentameric bundle

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Abstract The transmembrane domain of oligomeric protein Vpu encoded by HIV-1 has been studied by means of a molecular dynamics simulation. A pentameric bundle of unconstrained helices (residues 6–28 of Vpu) with a water filled pore was initially assembled in a membrane mimetic octane/water system. This system was simulated, using the CHARMm19 and OPLS united atom force fields with no constraints at a temperature of 300 K and a pressure of 1 atm. For these forcefields and the initial conditions tested, the oligomeric bundle expelled most of the pore water molecules. The resulting bundle and residual waters adopt a conical structural motif with some resemblance to a potassium channel.

Key words: Human immunodeficiency virus-1; Vpu; Transmembrane helix; Ion channel; Molecular dynamics

1. Introduction

Vpu is an oligomeric protein encoded by HIV-1 and is present in the Golgi and endoplasmic reticulum membranes of infected cells. Vpu has two biological roles that are associated with two distinct domains within the protein. The C-terminal cytoplasmic domain is associated with the induction of CD4 degradation in the endoplasmic reticulum whereas the N-terminal transmembrane domain is associated with the release/secretion of the virus from HIV-1 infected cells [1]. Vpu has 80–82 amino acid residues (depending on the variant) and contains three helical subunits, one of which is a transmembrane helix (from residue 6 to 28) [2]. The transmembrane portion of the protein is believed to form an ion channel [3–5], which is selective for monovalent cations (Na^+ and K^+). However, it is still unknown how many transmembrane helices form the ion channel, or what role the other domain plays in the functioning of the ion channel. A recent paper by Grice, Kerr, and Sansom [6] has suggested that a pentamer of Vpu transmembrane sequences is most likely present in the Vpu oligomeric bundle. This conclusion was based on an estimate of the conductance of the channel from molecular dynamics (MD) simulations. However, as admitted by the authors, a number of approximations underlie the simulations and the model for estimating the channel conductance.

In the present study we have used MD to simulate the same Vpu^{6-28} peptide transmembrane portions and relaxed some of the assumptions that went into earlier work. The major differences in the present investigation are the removal of all structural constraints, and the inclusion of an explicit membrane-mimetic medium to solvate the bundle. With a more detailed simulation we investigate some specific aspects of the nature of the pore. Anticipating our results, we find that an unconstrained pentameric aggregate can associate to form a compact bundle with some of the gross features reported by Grice et al. [6], but the resulting bundle is not able to support a continuous water channel. However, in this passive state the bundle does exhibit a cone-line C-terminal region. Further, we find that the initial setup used by Grice et al., which likely influences the outcome of their MD simulation is not the optimal choice. Our findings raise some questions about the nature of the Vpu oligomeric bundle.

The paper is organized as follows: Section 2 described the simulation methods, Section 3 presents our results and Section 4 contains discussions and conclusions.

2. Methods

The initial configurations were obtained by setting up an idealized ion channel bundle similar to that of Grice et al. [6]. Each helix in the bundle was constructed as an ideal right-handed α-helix, and contained 23 amino acid residues (Ac-IVAIVALVVAIIIAIVVWSIVII-NH_2) with a total of 203 united atoms. Not all hydrogens in the protein were explicitly defined, since some of them were incorporated into united atoms. The helix residue numbering starts at 6 for consistency with the experimental work. The bundles were set up with 5-fold rotational symmetry along the long axis. Two different initial conditions were used: one where the serine, residue 24, pointed into the center of the pore, which is similar to Grice et al., and the other that placed the tryptophan, residue 23, in the pore.

All MD simulations were performed using the NPT ensemble (constant number of atoms, constant pressure and constant temperature) at a pressure of one atmosphere and a temperature of 300 K. The algorithms used to perform the MD included recent improvements to molecular dynamics integrators employing multiple time steps and extended systems [7,8]. Periodic boundary conditions were employed with the Ewald method to account for long-range electrostatic interactions. The real space summation was cut off at 10Å and a convergence parameter of 0.3 $Å^{-1}$ was found to be sufficient. The van der Waals interactions were cut off at 10 Å with long range corrections to the pressure and energies to account for this truncation [9]. For the constant pressure simulation the cell lengths were allowed to dilate along independent Cartesian directions while the cell angles were held fixed. This independent motion of the cell walls allowed the water and octane layer to relax laterally around the bundle. Independent variable cell lengths are important to prevent biasing the simulation towards a particular octane or water layer thickness [10].

The model employed for water was the so-called flexible SPC/E due to Toukan et al. [11]. The octane layer used to mimic the hydrophobic part of a lipid bilayer was modeled using the parameter set recommended by Siepmann et al. [12]. The potential between the octane and the atoms on the protein were site specific. They have been shown to reproduce experimental structural data for these types of interactions [10]. While the octane layer does not have all the characteristics of a lipid bilayer, it does have the correct qualitative features of the desired hydrophobic-hydrophilic interface.

Two different force fields, CHARMm19 [13] and united atom OPLS [14] were used to look for any bias from the potential function. These

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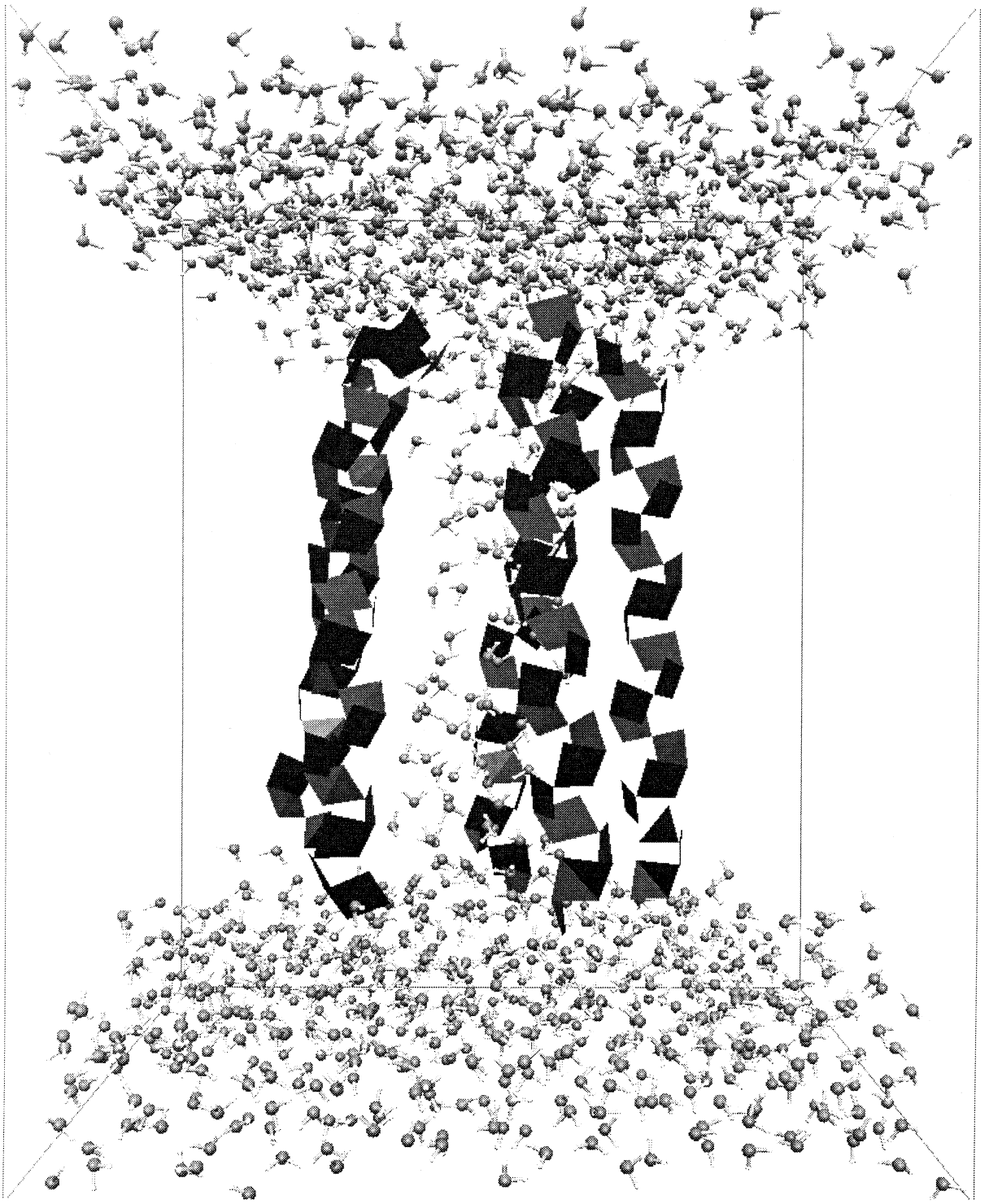

Fig. 1. A representative initial configuration of the pentameric bundle in a membrane mimetic octane/water system. The octane molecules, which make up the hydrophobic region, are not shown so that the water column is visible. The five peptide backbones are rendered as linked planes. In this initial configuration there are 214 water molecules within the pore. The C-terminal ends of the Vpu peptides are at the top.

united atom potentials, with less interaction sites, were chosen for computational efficiency. While these united atom force fields may not reproduce all the fine structural details, they are likely sufficient to give qualitative results and gross structural features [13], as required in the present study.

With the peptide centers of mass constrained, the bundle was annealed in vacuum to eliminate unphysical contacts between residues. The interior of the bundle was then solvated by inserting an equilibrated cylinder of water into the pore. Water molecules were removed from the pore if an atom of O or H was within 2 Å of any of the atoms of the bundle. The bundle, including the pore water, was then inserted into a 30-Å thick slab of octane and a slab of water 25 Å

thick was placed on top of the system. Solvating molecules were eliminated in the same manner as described above. The dimensions of the initial simulation cell were $45 \times 45 \times 65$ Å, which gives a density (0.9 g/cc) and is similar to physiological conditions. The composite system contained 5 helices (1015 united atoms), 196 octanes (1568 united atoms) and 1382 water molecules (4146 atoms) for a total of 6729 interacting sites.

Simulated annealing was performed on the entire system to eliminate any unphysical contacts between the helices and the solvent molecules. MD was then performed on the composite system until the potential energy, kinetic energy, volume, cell lengths and helical structure stabilized. For the four different systems, two force fields and two conditions the equilibration period lasted between 0.5 and 1.5 ns before stabilization occurred. This was considerably longer than the previous simulations that have been reported. MD runs of 1 ns were then performed from which the time averaged quantities were obtained.

It is important to note that no constraints were imposed on the system. Thus, unlike Grice et al. there were no 'bonds' between the helix to keep the bundle together. Also, there were no constraints on the diffusion of water molecules keeping them within the pore. There were no constraints on the bonds within Vpu segments, which might effect the low frequency vibrations of the molecules and inhibit conformational changes. Each helix was allowed to diffuse and rotate, and the bundle was followed for long enough times to adopt substantially different structures.

3. Results

A representative initial configuration of the system is shown in Fig. 1. We will describe the two simulations that employed the CHARMm19 potential in detail. The simulation with the OPLS force field gave essentially the same results. The first simulation had the serines (residue 24) in the pore, thereby allowing it to hydrogen bond with a water molecule. The second simulation had the tryptophans (residue 23) extending into the pore. All simulations had approximately 200 water molecules within the pore at the start of the calculations.

The average quantities reported below are a time average over the last nanosecond of simulation and of the four different calculations, which consisted of the two initial conditions and the two forcefields. The average length of channel is 33.9 Å, where the length is defined as the distance between the centers of mass of the two cap residues (ACE-NH_2). The value obtained is roughly consistent with a typical lipid bilayer spacing [15]. The average tilt of the Vpu peptides is 4.2 deg, where the tilt is defined as the average angle made by the largest component of the moment of inertia tensor of the bundle with respect to the normal of the octane-water interface. Thus, the helices and the entire bundle are nearly perpendicular to the interface. Also, there is no appreciable tilt angle between the helices themselves. The lack of super coiling was also found by Grice et al., and this observation is in contrast to other known ion channels and calculations [10,16]. The average dipole moment of each helix was 82 Debye, and with no tilt the total dipole of the bundle was about 5 times this amount. This large dipole might influence and attract ions into the pore region. This large dipole might also be expected to stabilize water within the pore, even though there are mostly hydrophobic residues lining the pore.

For the first simulation (CHARMm19 parameters with the serines within the pore), the volume and energies started to fluctuate around their mean values after 200 ps. During the next 500 ps approximately 80 water molecules were slowly expelled from the pore of the bundle. In the subsequent 500 ps, the bundle deformed so that one of the helices rotated to allow its tryptophan residue to point into the center of the pore, as shown in Fig. 2. The remaining four helices rotated such that the tryptophan extended to one of the neighboring helices. Over the next 200 ps, another 60 waters left the pore. The 'volume' that was initially occupied with water in the pore was now taken up by hydrophobic residues, most notable leucine (residue 12). Fig. 3 shows the system after 1.2 ns of simulation. This state, with no continuous water column, was stable over an additional nanosecond. Thus the final state of the pentamer no longer supports a continuous water column.

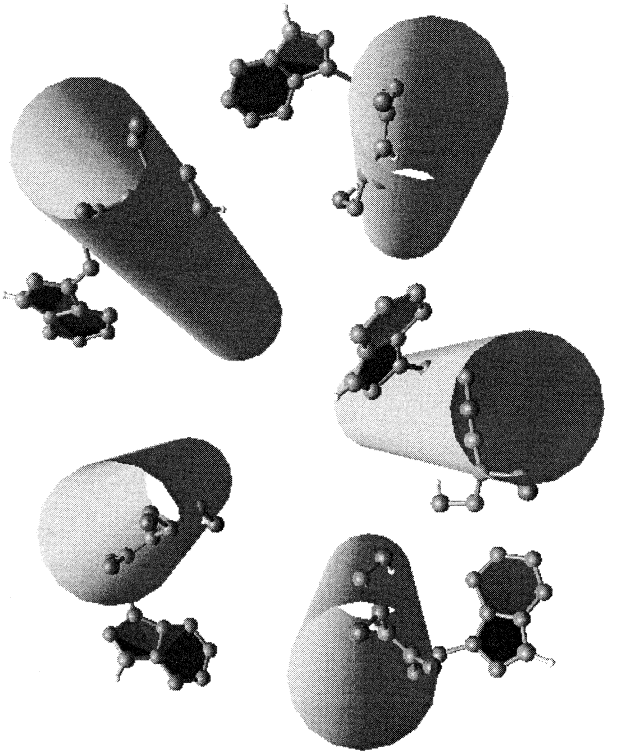

Fig. 2. A snap-shot after 1 ns taken from a simulation where the tryptophans were initially on the outside of the pore. The view is down the center of pore from the C-terminal end, with the water and octane molecules omitted for visual clarity. Only the serine and tryptophan residues are illustrated, with the tryptophan atomic planes shaded. The helices are represented as cylinders. A single tryptophan is within the pore water column. The other tryptophans are in contact with a neighboring helix.

For the second simulation, which employed CHARMm19 parameters and tryptophans initially pointing into the pore, the volume and energies started fluctuating around their mean values after 100 ps. Like the first simulation, water was expelled from the bundle over the next 500 ps leaving 60 waters within the pore. The water column was broken in a similar manner to the first simulation. However, after 100 ps with no continuous water column, a single octane molecule penetrated into the bundle and blocked the pore within the hydrophobic region, between residues 8 and 18. From Fig. 4 it is seen that helices rotated such that two of the tryptophans resided between the helices. However, this rotation did not place the tryptophans completely within the hydrophobic region. The presence of tryptophans in an amphiphilic region is a common feature of all our calculations. It is interesting to note that two of the helices were closely linked through a tryptophan-tryptophan interaction, which persisted for the length of the calculation.

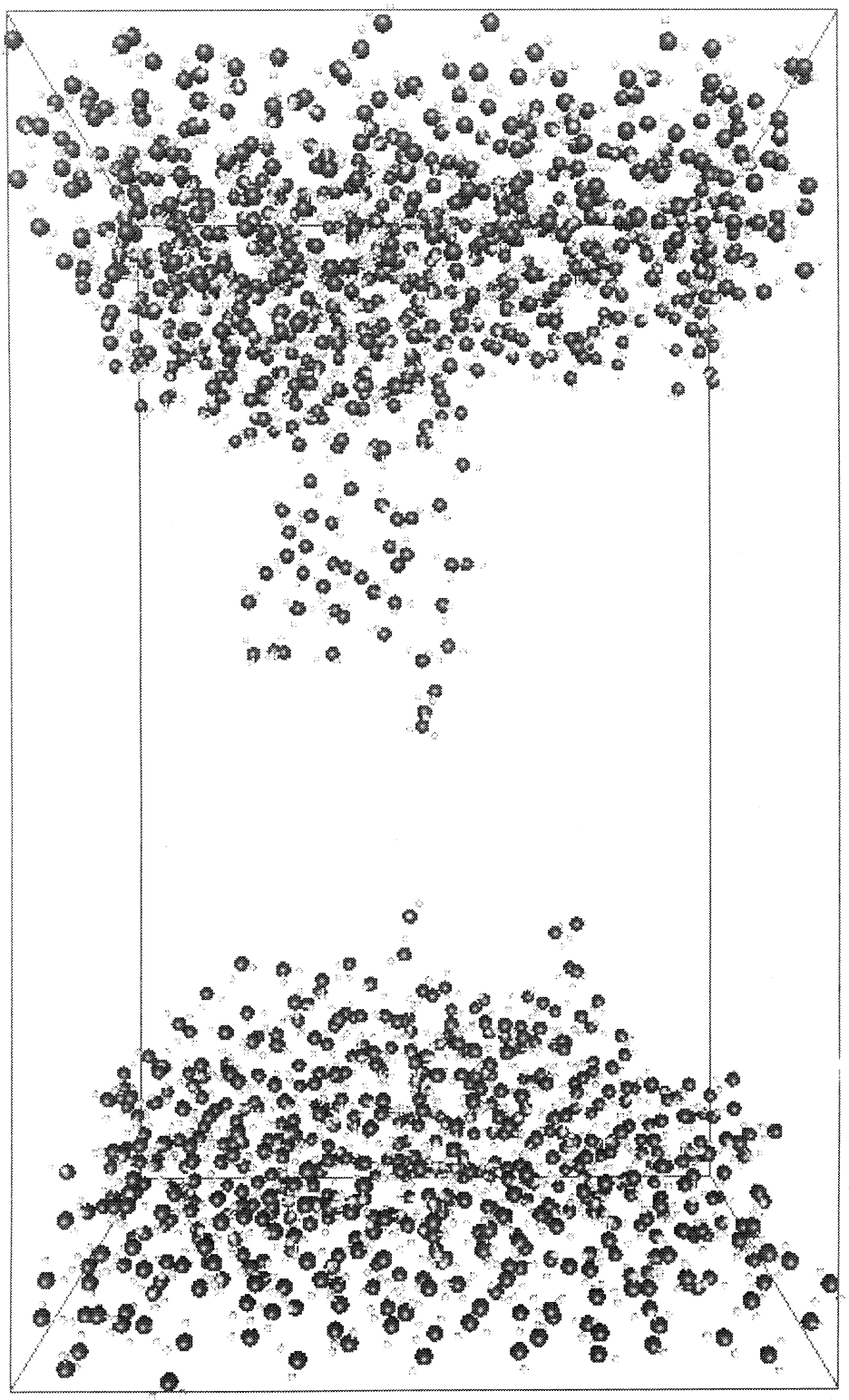

Fig. 3. A representative final configuration, showing only the water molecules. The column of water no longer extends through the hydrophobic region. There is significant solvation near the top of the bundle in the region of the tryptophan and serine residues, but no water molecules are observed in the region around the hydrophobic residues 8–18. This result is characteristic of all four of our MD calculations.

In all four MD runs, the pentamer was stable on the time scale of the simulation and showed no tendency to dissociate, shear or uncoil. Different initial conditions and parameters showed qualitatively similar results. All simulations showed removal of most of the water, such that there was not a continuous column of water through the hydrophobic region. In all cases at least one tryptophan rotated such that it resided in an amphiphilic region surrounded by water, octane and helix.

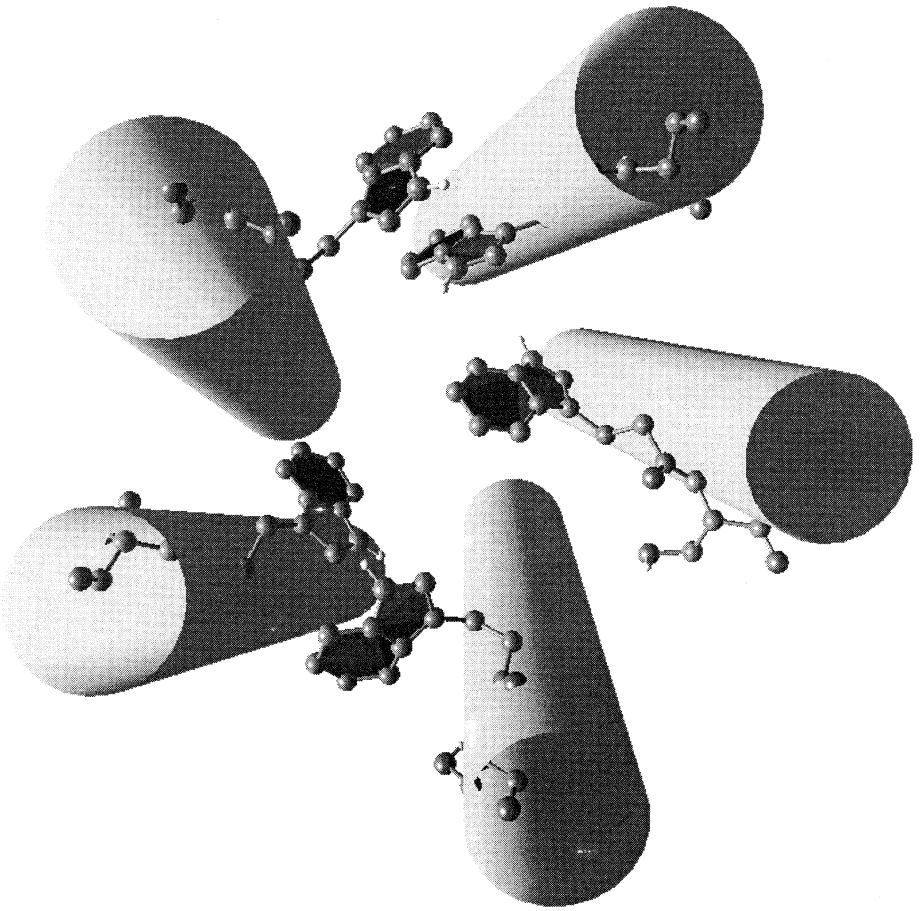

Fig. 4. A snap-shot, taken after 1 ns of MD simulation, looking down the pentameric bundle from the C-terminal end. The peptide backbones are drawn as cylinders. The tryptophan atomic planes are shaded, and the serine residue atoms are also shown. All the tryptophans were initially placed facing into the pore. After 1 ns two tryptophans showed a tendency to reside between the helices. This behavior was observed regardless of the initial conditions (see text).

Also, the initial pentameric symmetry was lost; all simulations adopted an oblong shape perpendicular to the pore. Also, the helices formed a conical shape along the length of the pore, with residual water at the C-terminal portion of the peptide.

4. Discussion and conclusion

The present results are very different from those reported by Grice et al., specifically the removal of structural constraints leads to no continuous water column through the membrane. At first sight, this suggests that there is no ion channel activity since there is not a continuous water channel through the bundle. However, the structure found recently for potassium channels [17] also has a region without a water column. Our calculations show that if the pentameric bundle does exist then it is not symmetric, and that at least one tryptophan residue is residing between helices. Also, we find that the bundle adopts a conical shape with water at the C-terminal portion, not a cylindrical shape as was initially proposed.

The equilibrium state of the bundle without a continuous water column is similar to the structure found recently for potassium channels [17]. The region with no waters for 15 Å is about the same length as the selectivity filter in the potassium channel, which also has no water in this region of the channel. Another feature, common to our calculation and the potassium channel, is that there is a hydration pocket which extends half way through the hydrophilic region. This would allow for ions to penetrate into the hydrophobic region without crossing a large energy barrier.

As in earlier work the approximations, assumptions, and limitations need to be considered. We have assumed many things in the chosen initial conditions. While we have used a pentamer, this is not necessarily the number that is actually present in the experiments. There is no real justification for this to be the case and our calculations cannot exclude that a larger number of helices might be needed if Vpu is to act as an ion channel. Another possible problem with our calculation is that we leave off the first 5 amino acids used by Schubert et al. [3]. This was done to compare with earlier calculations and for computational efficiency. Initially, we thought that these residues would not influence the pore structure greatly. However, with hindsight the inclusion of these few residues (Met-Glu-Pro-Ile-Gln-), which are largely hydrophilic, could alter the pore structure and dynamics. Specifically, the Gln might be solvated in such a way as to allow water to reform a pore. The inclusion of these five residues, which has the sequence that showed ion channel activity is currently under investigation.

Another limitation in our model is the use of octane as a bilayer mimetic. While this hydrophobic region does possess many of the salient properties of a bilayer, the interface is

different from that of a true lipid bilayer. This might influence the pore radius at the interface of the bilayer as well as the dynamics of the bundle. It would be very difficult for a portion of a lipid to block the channel as we observed in one of our simulations. Further, we do not have a voltage, as formed in the conductance experiments. The inclusion of a field across the membrane is also part of our ongoing research [18].

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