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ARTICLE *in* THE JOURNAL OF PHYSICAL CHEMISTRY B · JANUARY 2009

Impact Factor: 3.3 · DOI: 10.1021/jp808417a · Source: PubMed

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2009, *113,* 6–8
Published on Web 12/11/2008

Spontaneous Formation of a Barrel-Stave Pore in a Coarse-Grained Model of the Synthetic LS3 Peptide and a DPPC Lipid Bilayer

P. Gkeka and L. Sarkisov*

Institute for Materials and Processes, University of Edinburgh, Edinburgh EH9 3JL, United Kingdom

Received: September 22, 2008; Revised Manuscript Received: November 3, 2008

We perform long-time-scale coarse-grained molecular dynamics simulations of the synthetic amphiphilic LS3 peptide interacting with a DPPC lipid bilayer. Our studies show that within several microseconds, the peptide assembles in a trans-membrane barrel-stave pore. The pore consists of six peptides and has an inner diameter of about 5.2 Å, which is comparable to earlier experimental and more detailed atomistic studies. Other structures such as three-, four-, and five-member bundles are also observed.

Introduction

Molecular modeling of peptide−membrane interactions on a detailed atomistic level has been of growing importance in our efforts to explain and understand antimicrobial defense mechanisms, cell penetration by peptides, the functioning of ion channels, membrane fusion, and many other processes.

Many of these phenomena require a description operating over long time and length scales (microseconds and tens of nanometers). Traditionally, this has been a fundamental challenge for atomistic computer simulations, typically limited to a few thousand molecules and tens of nanoseconds. However, recently, the possibility to construct this description emerged from new efficient coarse-grained approaches, where the idea is to systematically reduce the level of detail in the way the system is represented and therefore to increase the time/length scale of the simulation. A number of models and approaches have been suggested over the years, and we refer the reader to an excellent recent review by Venturoli and co-workers.[1] One particularly notable model was proposed by Marrink and co-workers where, on average, four atoms (excluding hydrogen) are represented as one effective bead.[2] It has been shown in a series of studies that the proposed model accurately describes formation, properties, and phase transitions of lipid bilayers as well as micellar and vesicle behavior, clearly demonstrating that fairly long time and length scales are within the scope of the model.[3−5] Recently, Bond and Sansom have extended the model of Marrink and co-workers to investigate the insertion and assembly of Glycophorin A (GpA) and OmpA proteins and a number of peptides in a lipid bilayer.[6,7] In the case of GpA, which is an α-helix of 23 residues, they observed insertion and dimerization of the peptide within the bilayer, in agreement with experiments and more detailed atomistic simulations.[6] In another example, Shih et al. extended the model of Marrink and co-workers to study lipoprotein particles.[8] Recently, a new version of the force field proposed by Marrink and co-workers, MARTINI, has been developed with a focus on bimolecular interactions and peptides.[9,10] This force field features a larger number of bead types and interactions, optimized to reproduce some key properties of amino acids, such as oil/water partition coefficients. It has been shown that the proposed model accurately captures peptide−membrane interactions for several trans-membrane peptide families.[10] Furthermore, the model correctly reproduces the formation of a toroidal pore by magainin-H2, confirming earlier atomistic simulations.[11]

Clearly, this coarse-grained approach is very promising as it offers a possibility to underpin many intriguing aspects of peptide−membrane interactions on the molecular level. Therefore, it is important to carefully probe its scope and generality in application to other families of peptides and to other mechanisms of interactions. In particular, it has been theorized that interactions of α-helical peptides with lipid membranes can be explained in terms of the relative distribution of hydrophilic and hydrophobic groups in the helix. Depending on the location (or concentration) of hydrophobic groups, one may observe the formation of a transient barrel-stave or a toroidal pore formed by several peptides; a peptide may prefer the interfacial region of the membrane or be obliquely inserted.[12] Clearly, being able to systematically capture these scenarios provides a robust test to any coarse-grained model aimed to reproduce peptide−membrane interaction mechanisms.

In this Letter, we extend the coarse-grained approach of Marrink and co-workers to a specific class of pore-forming peptides, LS3. LS3 is a synthetic, amphiphilic peptide designed to imitate properties of α-helices forming membrane ion channels. Specifically, the 21 residue peptide has a repeating $(LSSLLSL)_3$ motif with hydrophobic (leucine, L) residues and hydrophilic (serine, S) residues forming two parallel bands on the surface of the helix. Indeed, a number of studies confirm that this peptide spontaneously assembles in an ion conduction bundle within a lipid membrane.[13−17] The bundle is composed of approximately six trans-membrane α-helices. Each helix is oriented so that most of the polar residues (S) lay inside of the pore, whereas apolar residues face the hydrophobic medium of the bilayer. It has been also noted that the applied trans-membrane voltage significantly enhances pore formation due to asymmetric charge distribution within the helix (the N-terminus is positive and the C-terminus is negative). Bond and

* To whom correspondence should be addressed. Tel.: +44 131 650 4862. E-mail: Lev.Sarkisov@ed.ac.uk.

10.1021/jp808417a CCC: $40.75

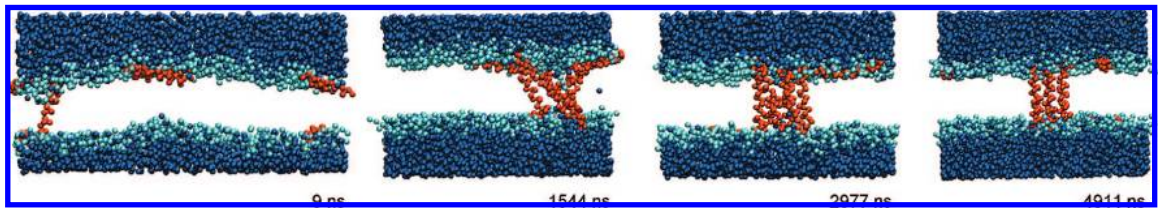

Figure 1. Six LS3 peptides form a barrel-stave pore within a DPPC lipid bilayer. Water is shown in blue, and the lipid heads are shown in cyan. The hydrophobic tails are not shown for clarity. For the peptides, only the backbone beads are shown (orange). The beads are not shown to scale.

Sansom studied a coarse-grained model of the LS3 peptide and observed that a single peptide prefers to be at the surface of the bilayer, in agreement with the experimental work.[7]

Results and Discussion

In this work, all species are described using the MARTINI force field, and we refer the reader to the original publications for the details of parameters.[9,10] We consider a system consisting of 3228 water particles, 256 DPPC (dipalmitoyl-phosphatidylcholine) lipids, and from 6 to 12 peptides. In the coarse-grained representation, each peptide consists of 42 effective beads, with 9 polar beads of serine side chains forming a fairly ordered vertical strip on the surface of the helix. The simulation protocol consists of two stages. In the first stage, we perform a molecular dynamics simulation of water and lipid components, resulting in the formation of a well-defined lipid bilayer. In the second stage, once the bilayer is formed, several peptides are placed at random locations throughout the system, and energy minimization is performed to relax possible unphysical contacts. This is followed by a long NPT molecular dynamics simulation (up to 12 μs), and the evolution of the system is observed via computer visualization and various properties of the system. All simulations in this study are carried out at $T = 323$ K, $P = 1$ bar, with time step of 25 fs, and with a Berendsen thermo- and barostat.[18] Both Lennard-Jones and Coulombic interactions are cut off and shifted at 1.2 nm, similar to the protocol employed by Monticelli et al.[9] All simulations are performed using the Gromacs simulation package.[19] System configurations are visualized and explored using the visual molecular dynamics (VMD) software.[20]

Figure 1 shows a number of system configurations observed at different times as the simulation progresses. During this process, several peptide complexes are observed, including dimers and trimers with some of the peptides at the surface of the bilayer and the others inside of the bilayer at an oblique angle. The actual pore formation seems to be initiated when two or more peptides adopt a proper trans-membrane orientation. The two final configurations show six peptides forming a trans-membrane pore. From the configurations of the lipid molecules in the vicinity of the bundle, this structure can be classified as a barrel-stave pore. Figure 2 shows in detail characteristics of the pore formed by six peptides. Its internal diameter is about 5.2 Å (the diameter of a sphere that fits inside of the pore), whereas in earlier experimental and simulation studies, this parameter was estimated at <8 Å.[13,14] It is further important to note that the pore is filled with a few water particles, although they are not shown in Figure 2 for reasons of clarity. Figure 2 also shows the orientation of the hydrophilic groups, corresponding to serine (S) within the pore, and it is quite evident that most of them face the interior of the bundle. Interestingly, this pore forms in the absence of a trans-membrane potential, and naturally, there is no preferred orientation of the peptides within the bilayer. However, the impact of the applied electrostatic field will be investigated in future work. Computer visualization of the dynamics of the system shows that the mobility of the peptides within the bundle is quite limited; however, the bundle as a whole is able to freely move within the bilayer plane.

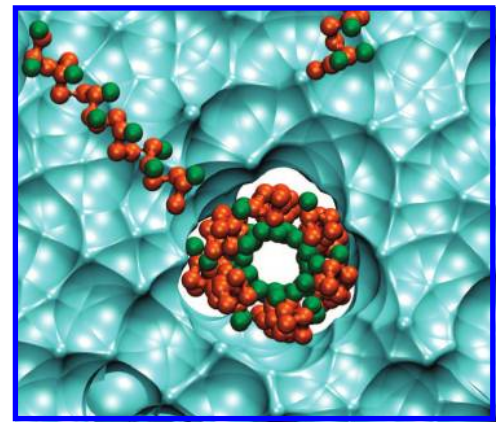

Figure 2. Top view of the pore formed by six LS3 peptides. The phosphate groups of the lipid molecules are shown as the cyan surface. The backbone beads of the peptides are shown in orange, and polar beads, corresponding to serine (S), are shown in green. Other groups and beads are not shown for clarity. The beads are not shown to scale.

We repeat the simulation several times and with different concentrations of the peptide to confirm the robustness of the model. In several cases, we observe spontaneous formation of pores with three, four, five, or six peptides participating in the bundle. It is also worthwhile to mention that we are not able to reproduce this behavior with the model of Bond and Sansom and several of its variants, with the peptides always remaining in the interfacial region of the bilayer. We believe that this disagreement between the models could be related to a somewhat different representation of the residues in the vicinity of the helix termini in two models. In the MARTINI force field, the regions close to the termini are more polar compared to the Bond and Sansom approach. In principle, this should make the insertion of a peptide more difficult, and indeed, with a single peptide at the lipid interface, we never observe an insertion. However, the presence of other peptides seems to facilitate the insertion. In this process, transient associations between peptides play a role, and thus, the polarity of the peptide is also important. We currently investigate this effect in more detail.

Finally, there is an important concern related to whether it is appropriate to apply a coarse-graining procedure to the solvent (water) where several solvent molecules are represented as one effective bead.[21]

Here, we adopt a simplified view that the coarse-grained water beads in this model represent some generic polar solvent. This study provides strong evidence that, within the qualitative scope, the employed model can be used to reveal, classify, and explain

possible modes of peptide−membrane interactions and guide the design of synthetic peptides with tailored antimicrobial, cell penetrating, and other useful characteristics.

Acknowledgment. The authors would like to thank the Institute for Materials and Processes at the University of Edinburgh for financial support and the Edinburgh Compute and Data Facility for the computational resources.

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JP808417A