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Molecular dynamics methods to predict peptide locations in membranes: LAH4 as a stringent test case

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ABSTRACT

Determining the structure of membrane-active peptides inside lipid bilayers is essential to understand their mechanism of action. Molecular dynamics simulations can easily provide atomistic details, but need experimental validation. We assessed the reliability of self-assembling (or "minimum-bias") and potential of mean force (PMF) approaches, using all-atoms (AAs) and coarse-grained (CG) force-fields. The LAH4 peptide was selected as a stringent test case, since it is known to attain different orientations depending on the protonation state of its four histidine residues.

In all simulations the histidine side-chains inserted in the membrane when neutral, while they interacted with phospholipid headgroups in their charged state. This led to transmembrane orientations for neutral-His LAH4 in all minimum-bias AA simulations and in most CG trajectories. By contrast, the charged-His peptide stabilized membrane defects in AA simulations, whereas it is located at the membrane surface in some CG trajectories, interacting with both lipid leaflets in others. This behavior is consistent with the higher antimicrobial activity and membrane-permeabilizing behavior of the charged-His LAH4. In addition, good agreement with solid-state NMR orientational data was observed in AA simulations.

PMF calculations correctly predicted a higher membrane affinity for the neutral-His peptide. Interestingly, the structures and relative populations of PMF local free-energy minima corresponded to those determined in the less computationally demanding minimum-bias simulations.

These data provide an indication about the possible membrane-perturbation mechanism of the charged-His LAH4 peptide: by interacting with lipid headgroups of both leaflets through its cationic side-chains, it could favor membrane defects and facilitate translocation across the bilayer.

1. Introduction

Several peptides exert their functions by interacting with cell membranes. Based on their effect, these membrane-active peptides are usually divided in different classes. Host-defense peptides are a heterogeneous class of amphipathic oligopeptides, which kill pathogens, and even cancerous cells, mainly by inducing leakage of their cell membranes through physical interactions with the lipid bilayer, making these molecules promising compounds to fight drug-resistant bacteria [1–3]. Cell-penetrating peptides (CPPs) are used to deliver therapeutic molecules (nucleic acids, drugs, imaging agents) to cells and tissues in a nontoxic manner [4,5]. In the case of amyloid peptides the mechanisms of aggregation and toxicity are not fully understood, but a common property of these peptides is their ability to interact with lipid bilayers, thereby disturbing membrane integrity. Lipid bilayers can also act as conformational catalysts, favoring protein misfolding and aggregation [6]. Fusion peptides are segments of viral proteins, or model oligopeptides, with the ability to facilitate the merging of two apposed lipid bilayers, a fundamental event in many biological processes [7]. Finally, other peptides are able to recognize membrane regions with a specific curvature, or to deform lipid bilayers [8]. Notably, many peptides exhibit various functionalities and the separation into distinct classes is not always obvious [9].

The molecular details of the mechanism of action of many of these systems are still debated. This deficiency is due mainly to the difficulties involved in the application of atomic-resolution structural techniques (X-ray crystallography and NMR spectroscopy) to membrane systems.

Abbreviations: PMF, potential of mean force; AAs, all-atoms; CG, coarse-grained; MD, molecular dynamics; CPP, cell-penetrating peptide; FF, force-field; ATR-FTIR, attenuated total reflection Fourier-transform IR; OCD, oriented circular dichroism; TM, transmembrane; LAH4-c, charged His LAH4; LAH4-n, neutral His LAH4; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; POPS, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-L-serine; SW, standard water; PW, polarizable water; COM, center of mass; WHAM, weighted histogram analysis method

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Although significant advances are being achieved in these areas [10,11] determination of the structure, position and orientation of peptides and proteins in membranes is still a challenge, in particular as the outlines of the lipid bilayers in or from high-resolution structures often remain purely speculative. For this reason, alternative approaches are of particular interest to study peptide–lipid interactions.

Molecular dynamics (MD) simulations can provide atomic-level data on the structure and dynamics of peptide–membrane systems [12,13]. However, they might be affected by the approximations used in the computation of the trajectories or suffer from sampling and convergence problems due to the limited length of the simulations, typically in the 0.1–1 μs time-range. For instance, the simplest, brute force approach follows the motions of all atoms in the system without any external perturbation ("unbiased simulations"), starting with a preformed bilayer and with the peptide in the water or in the membrane phase. In these cases, relaxation to the minimum free-energy configuration and peptide conformation often does not take place during accessible time-scales, due to the relatively viscous and ordered membrane environment [14–17]. Therefore, the "unbiased" attribute might be misleading for these simulations of membrane systems, since the final results are significantly affected by the starting conditions (even though technically the term "unbiased" refers to the absence of external perturbations to the system).

A common method to increase the accessible simulation time is based on the so-called coarse-grained (CG) force fields (FFs) [18]. In CG simulations, groups of atoms are treated as a single particle (bead), thus reducing the degrees of freedom of the system. The loss of atomic details is balanced by an increase in sampling of up to 4 orders of magnitude, in comparison with AA simulations [19]. For this reason, CG FFs, and particularly MARTINI, are widely applied to peptide–membrane systems, although the approximation used for water molecules has been consistently shown to disfavor the formation of membrane defects or pores [20]. In addition, also for these kinds of FFs, "unbiased" simulations starting from preformed bilayers might fail to determine the correct peptide position/orientation in the membrane, because the system is caught in a local free-energy minimum [21].

To solve problems related to the slow relaxation of membrane systems, we [2,22,23] and others [18,24–28] have used an approach that we termed "minimum bias", since it minimizes the effect of the initial configuration on the final results. In this method, which has been applied to both AA and CG FFs, the simulation is started from a random mixture of peptide, lipids and water, and the bilayer forms spontaneously in 50–100 ns. During this self-assembly process, the system is much more fluid than a fully formed bilayer, especially in the first stages of the simulation. This ensures that the peptide can experiment different environments in a relatively short time, and, as a consequence, it is more likely to find its minimum free energy configurations. Notably, this approach is similar to many biophysical experiments, such as solid-state NMR, attenuated total reflection Fourier-transform IR (ATR-FTIR) or oriented circular dichroism (OCD), where membranes are formed from lipid–peptide mixtures completely dissolved in organic solvents [29].

Another method to improve sampling of the system configurations involves the addition of constraints to pull the peptide from the water solution to the center of the bilayer. By performing several independent simulations with the peptide placed at different depths, it is possible to determine the potential of mean force (PMF), or free-energy profile, as a function of peptide position in the membrane. PMF calculations have been successfully applied to determine the position and conformation of peptides in lipid bilayers [17,30–32]. In addition, PMF analyses give a picture of the whole insertion pathway, including the configurations corresponding to the potential energy barriers to the insertion, or to local minima. However, the need to attain an equilibrated system at each depth of peptide insertion [32] makes PMF calculations rather demanding, so that CG FFs are often used to reduce the computational costs [21,33,34].

In this article we aim to assess the reliability of the "minimum bias" (AA and CG) and CG PMF approaches, by comparing their results with experimental data. To this end, an ideal test case is provided by the designed peptide LAH4, which exhibits both antimicrobial and cell-penetrating activities [35,36]. Its sequence (KKALLALALHHLAHLALH LALALKKA-NH_2) comprises four histidines in the central part, which in micelles exhibit pKa values between 5.4 and 6.0, so that the total peptide charge can be tuned in a pH-dependent manner [37]. NMR, ATR-FTIR and CD experiments demonstrated that LAH4 is predominantly helical when membrane bound, with an amphiphilic distribution of the His and Leu/Ala side-chains [38–40]. In POPC membranes, the peptide helix changes its orientation together with the protonation state of the His residues, going from a prevalently in-plane to a transmembrane (TM) arrangement when the pH increases from acidic to neutral/basic values [37,38,41].

Although some previous studies compared solid-state NMR experiments with peptide orientations independently predicted from MD simulations [42,43], this is the first article focusing on a single peptide that can sample the different positions/orientations usually observed for membrane-active peptides, simply by varying its protonation state. While this study was in progress, a simulation of LAH4 interaction with membranes was published [44]. However, in that case the simulations were started with a preformed bilayer and the peptide in the water phase, and LAH4 remained associated to the membrane surface, mostly above the phospholipid headgroups, likely due to the problems of convergence affecting this kind of "unbiased" simulations (see above). Our study also offers the additional benefit of comparing different computational approaches (AA and CG minimum bias simulations and CG PMF calculations), so that the advantages and limitations of each method can be discussed. Finally, the computational results can be used to provide an atomic level insight into the mechanisms of membrane activity of LAH4. This peptide has a strong bactericidal activity both at acidic and neutral/basic pH values, but it is more active and membranolytic when its His residues are charged [36,39]. A stronger lytic activity of the charged-His peptide state (LAH4-c, henceforth) has been observed also in model POPC bilayers, even though the neutral-His peptide (LAH4-n) has a higher membrane affinity [39,45].

LAH4 exhibits also an interesting ability to facilitate the entry of nucleic acids into cells, which is correlated with the change in endosomal pH during the transfection process [35,46]. Interaction of DNA with charged peptide residues favors its condensation, while protonation of the His side-chains in the acidic environment of endosomes, and thus activation of the peptide membrane permeabilization activity, favors the escape of nucleic acids to the cytosol [35,47]. However, notwithstanding many studies and several successful practical applications of LAH4 and its analogues in transfection [48,49], the molecular details of their membrane-perturbing activities still need to be clarified.

2. Methods

2.1. AA simulations

AA simulations of LAH4 were performed using the "minimum-bias" method, as previously described [22,24], except for the details reported below. LAH4 was modeled into an α-helix structure and placed at the center of a 9 × 9 × 9 nm box. POPC was selected for forming the membrane since this is the lipid used in the experimental determination of peptide orientation [37,38]. 128 POPC molecules with different conformations and 7500 water molecules were randomly added into the box. Finally, 5 or 9 chloride ions (depending on the protonation state of LAH4 His residues) were introduced in replacement of water molecules to neutralize the system. Similarly to our previous studies of peptide–membrane systems [2,13,22],simulations were performed with the ffgmx force field, implemented with the previously reported parameters for POPC [50], using the GROMACS 4.5 software package [51]. A control box without the peptide and a total of five LAH4 containing

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