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Molecular dynamics simulations of histidine-containing cod antimicrobial peptide paralogs in self-assembled bilayers

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ABSTRACT

Gaduscidin-1 and -2 (GAD-1 and GAD-2) are antimicrobial peptides (AMPs) that contain several histidine residues and are thus expected to exhibit pH-dependent activity. In order to help elucidate their mechanism of membrane disruption, we have performed molecular dynamics simulations with the peptides in both histidine-charged and histidine-neutral forms, along with 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) lipid molecules. The simulations employed GROMACS software and an OPLS-AA force field. Initially, the peptide and lipids were placed randomly in the simulation box and then were allowed to self-assemble. The results demonstrated a marked preference for the regions of the peptides that contain sequential pairs of histidine residues to associate closely with bilayer pores. This preference is observed even when the histidines are in their uncharged form. It appears that the relative compactness and rigidity of histidine pairs require the more aqueous and disordered environment of the pores to satisfy hydrophilic interactions. The final peptide structures exhibited a wide variety of structures and topologies, with the most helical structures positioning most parallel to the bilayer surface and the less ordered structures interacting more closely with the pore. Thus, the results give atomistic insight into those models of AMP mechanism that promote the importance of structural heterogeneity and imperfect amphipathicity to AMP activity and selectivity.

1. Introduction

Antimicrobial peptides (AMPs) play an important role in the immune systems of a wide variety of organisms, from humans to fish to insects. AMPs are generally amphipathic and cationic in nature (1), and consequently have a propensity to interact with lipid bilayers. Much of the research into the mechanism of AMPs has focused on their interaction with membranes, either as their direct mechanism of killing, or as a means of getting inside the cell to disrupt intracellular targets (2–5). Besides their natural roles in innate immunity, AMPs are also being investigated as potential therapeutics for conditions such as drug resistant infection (6–8) and cancer (9–11).

Many AMPs exhibit a degree of specificity and can kill pathogens at concentrations that do not harm host cells. At least some of this specificity is believed to derive from their cationic nature which provides for stronger interactions with, for example, bacterial or cancer cell membranes, which are generally more anionic in character than mammalian host cells (12,13). Nonetheless, one major barrier to using AMPs as drugs is that at high concentrations, they can kill not just the target cells but the healthy host cells as well (14,15).

One way of controlling the specificity and activity of AMPs is via controlling their charge. This is particularly applicable to peptides that contain the amino acid histidine, which is generally uncharged at neutral pH but tends to become positively charged at mildly acidic pH. This pH sensitivity of histidine-containing AMPs can provide a "pH switch" to activate them in lower pH environments (16–19). Acidic pH activation of AMPs may play a role in the endogenous functioning of AMPs; for example, acidic pH is important in skin immune defense (16,20). Moreover, there are intriguing possibilities to employ pH-activated AMPs in exogenous applications, such as the treatment of cancer (9,10) as the environment around tumors is usually acidic (17,21). Thus, histidine-containing AMPs are excellent candidates as therapeutics because they are likely to be much more active in the vicinity of the tumor than elsewhere in the body. Indeed, replacement of arginines and lysines with histidine in the AMP K6L6 was shown to make the peptide more specific; systemic injection of the modified peptide inhibited tumor growth in mice with reduced systemic toxicity compared to the parent peptide (17).

Abbreviations: GAD, Gaduscidin; AMP, antimicrobial peptide; POPC, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine; GROMACS, GROningen MAchine for Chemical Simulations; SPV, Swiss PDB Viewer; DSSP, Define Secondary Structure of Proteins; VMD, Visual Molecular Dynamics; OPLS-AA, Optimized Potentials for Liquid Simulations-All Atom

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At least three different mechanisms have been suggested for the altered activity of histidine-containing AMPs that is observed when histidines become more positively charged at lower pH. 1) Membrane binding: Kacprzyk et al replaced the lysine and arginine in synthetic AMP sequences with histidines to produce peptides that were only active under acidic conditions (16). The differences in activity corresponded well to observed differences in membrane binding. 2) Membrane penetration: Kharadia et al also replaced the lysine and arginine residues in lytic peptides with histidines. They found that the novel peptides were much more selective for bacteria over host tissue cells and attributed the increase in activity at lower pH not to changes in membrane binding, but to increased membrane penetration (22). 3) Changing peptide structure: This mechanism for pH-dependent activity in histidine-containing AMPs was suggested by detailed studies of the synthetic peptide LAH4, for which pH alterations induce changes in the peptide structure. At neutral pH a long helix is formed, which associates with the membrane in a planar manner. At slightly acidic pH, a hinge disrupts the contiguous helix structure, possibly due to electrostatic repulsion between adjacent histidines (23).

We were interested in exploring, at an atomic level, the role of histidines in AMP-membrane interactions. Of particular interest were histidines in natural sequences as opposed to the synthetic sequences that have been the subjects of most mechanistic studies of histidine-containing AMPs so far. Additionally, we wanted to probe the potential significance of histidines that appear in sequential pairs in the sequence, as opposed to histidines flanked solely by non-histidine residues. The peptides employed in the study, GAD-1 and GAD-2, are derived from codfish sequence libraries (24–27). They are paralogs, i.e., related genes found in the same organism, and their comparison has potential to illuminate the role of histidine pairs in evolutionarily tuned structure–function relationships. GAD-1 and GAD-2 are members of the piscidin family of helical AMPs. Piscidins have been subjects of several structure–function studies, although not studies that address pH-dependent behavior and mechanisms (28–37). GAD-1 (FIHHIIGWISHGVRAIHRAIH-NH_2) has 5 histidines, two of which appear in a pair and three of which appear singly. GAD-2 (FLHHIVG LIHHGLSLFGDR-NH_2) has 4 histidines, which appear in two sets of histidine pairs. We studied these peptides in histidine charged forms, denoted as GAD-1p and GAD-2p, as well as in the histidine neutral form (GAD-1, GAD-2). All-atom molecular dynamics simulations with these 4 peptides along with POPC lipids were performed in order to reveal atomistic details of their lipid interactions. As detailed in the Methods and Discussion sections, with our system setup, pores form even in the absence of peptide, and thus our study does not provide a kinetic picture of how the peptides bind to membranes and induce pore formation, but rather provides details of the peptide/lipid interactions. One of our key findings was that histidine pairs are more likely to be found closely associated with the pore than in the more ordered, planer region of the lipid bilayers.

2. Methods

Our approach followed the method of Salgado et al. (38) who start unassembled lipid molecules in random positions with a single peptide among the lipid molecules. This method allows the system to freely assemble into a peptide–bilayer complex, thus avoiding any artifacts that might result from, for example, introducing the peptide into a pre-assembled bilayer after removing lipids "by hand". The main difference in our implementation of this approach is that, rather than employing a script to randomize the position of the lipids, we randomized the system by simulating at high temperature, 1400 K. This modification provided ease of implementation, in particular by allowing for an initial volume not too much larger than the final one, while avoiding truly unphysical interactions.

Salgado et al. (38) found that the method produced expected outcomes in terms of the location and orientation of the peptide with respect to the membrane, i.e., a hydrophobic peptide ended up in a transmembrane configuration, while a more amphipathic peptide ended up on and parallel to the membrane surface. This lends confidence that the assembled structures reflect low free energy states of the equilibrium system. By contrast to these two simpler cases, imperfectly amphipathic peptides, such as the ones we are studying, may possess many different configurations of similar free energy. To address this, for each system composition, we performed four independent simulations of the self-assembly process. While computational resources limited us to this small number, it did provide sufficient sampling for at least a semi-quantitative characterization of the differences between the paralogs in their charged and uncharged forms. Additionally, simulations were carried out in the absence of the peptide to control for the effect of the peptide on bilayer formation.

The systems consisted of 128 POPC lipid molecules (6656 atoms), a single peptide (~350 atoms) and approximately and no less than 7360 water molecules (roughly 37,000 atoms in total) (Table 1). This number of lipid molecules provided a large enough bilayer to accommodate the peptide in the presence of a pore. The amount of water was chosen to allow for sufficient space to prevent periodic boundary conditions from permitting the peptide to interact unphysically with both leaflets through water. The number of water molecules employed is somewhat larger than the number employed in other studies (38–40).

The initial alpha-helical peptide structures were generated using Swiss PDB Viewer (SPV) (41–44). The C-terminus was amidated with an NH_2 group to be in line with ongoing experimental work. The peptide was placed in a cubic simulation box of side length 8 nm along with the 128 POPC molecules, which were initially arranged in a bilayer configuration taken from (45) (Fig. 1A). Sufficient Cl^- counterions were added to ensure overall charge neutrality: 3 ions for GAD-1, 8 for GAD-1p, 1 for GAD-2 and 5 for GAD-2p. For histidines in GAD-1p and GAD-2p, the protonated form of histidine was used.

GROMACS version 4.5 was used for the simulations (46). We employed a version of the all atom Optimized Potential for Liquid Simulations (OPLS-AA) force field (47,48), adapted for POPC lipid molecule properties (49).

As the first step in generating randomized starting configurations for the self-assembly process, we carried out a simulation of the system comprising the peptide, lipid bilayer and counter ions in the canonical ensemble at T = 1400 K for 2 ns, still within a cubic box of side length 8 nm, while restraining the position of all the peptide atoms to preserve its helical structure. We employed the modified Berendsen thermostat (*v-rescale* in GROMACS). Under these conditions, the lipid bilayer immediately disassembles, equilibrating rapidly to a highly mobile fluid of lipid molecules (Fig. 1B). In this regime, the root mean square displacement of a lipid molecule over 1 ns is approximately 10 nm.

Next, we added approximately 7500 TIP4P water molecules (50) to the simulation box and, after an energy minimization, continued running at 1400 K with the peptide still restrained. The root mean square displacement of lipid molecules over 2 ns was approximately 8.5 nm. During this run, we harvested four configurations, one every 2 ns, that served as independent starting configurations for separate realizations of the self-assembly process, which we label as A, B, C and D. This procedure for obtaining four independent configurations was carried out for each variant of the peptide (GAD-1, GAD-1p, GAD-2 and GAD-2p) as well as for a system without a peptide, which acts as a control. In total, there were starting points for 20 simulations of the self-assembly process.

Each self-assembly simulation began with a brief simulation of 100 ps under conditions of constant temperature and constant pressure, with the peptide unrestrained. The temperature was held constant at 310 K with the Nose–Hoover algorithm and a time constant of 0.1 ps. An isotropic pressure of 1 bar was maintained with the Parrinello–Rahman algorithm employing a time constant of 5 ps and compressibility of 4.5×10^{-5} bar^{-1}. This short simulation was sufficiently long to bring the density into a steady state (it does not continue to evolve in