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Molecular simulations suggest how a branched antimicrobial peptide perturbs a bacterial membrane and enhances permeability

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ABSTRACT

A covalently, branched antimicrobial peptide (BAMP) B2088 demonstrating enhanced antimicrobial effects and without additional toxicity when compared to its linear counterpart, has been developed. Atomistic molecular dynamics simulations have been used to investigate the mode of interaction of B2088 with model bacterial and mammalian membranes. These simulations suggest that both long-range electrostatic interactions and short-range hydrogen bonding play important roles in steering B2088 toward the negatively charged membranes. The reason why B2088 is selective towards the bacterial membrane is postulated to be the greater density of negative charges on the bacterial membrane which enables rapid accumulation of B2088 on the bacterial membrane to a high surface concentration, stabilizing it through excess hydrogen bond formation. The majority of hydrogen bonds are seen between the side chains of the basic residues (Arg or Lys) with the PO4 groups of lipids. In particular, formation of the bidentate hydrogen bonds between the guanidinium group of Arg and PO4 groups are found to be more favorable, both geometrically and energetically. Moreover, the planar gaunidinium group and its hydrophobic character enable the Arg side chains to solvate into the hydrophobic membrane. Structural perturbation of the bacterial membrane is found to be concentration dependent and is significant at higher concentrations of B2088, resulting in a large number of water translocations across the bacterial membrane. These simulations enhance our understanding of the action mechanism of a covalently branched antimicrobial peptide with model membranes and provide practical guidance for the design of new antimicrobial peptides.

1. Introduction

With the rapid increase in the incidence of bacterial resistance, the development of new and effective antibiotics is critical [1,2]. Antimicrobial peptides (AMPs) are one promising class of new and fast acting antibiotics that appear to demonstrate high antimicrobial activity, low toxicity and avoidance of resistance. They target bacterial membranes, which makes them very nonspecific, in contrast to traditional antibiotics, which target specific macromolecules in the cell. The latter are under evolutionary pressures and hence mutate, leading to resistance. In contrast membrane remodeling would be very costly for the bacteria and hence AMPs do not generate resistance easily. Despite numerous experimental and simulation studies on the mechanism of action of various AMPs [3–7], the underlying mechanism, namely how the AMPs perturb the membrane, remains enigmatic. A major reason has been attributed to the diversity of sequences and structures of AMPs, thus raising the possibility that they act in diverse ways [8,9], including through formation of pores and carpets [4,7,10–12]. Pore formation occurs as a result of the penetration of the AMP into the interior of the membrane and is thought to be the major mechanism underlying the activity of molecules such as protegrin, mellitin, magainin, etc. [13–16]. Depending on the interactions of AMPs with the membrane and the pore shape, two models of interactions have been put forward: (a) the barrel stave model which is characterized by the interactions between the hydrophobic part of the peptide and the hydrophobic region of the lipid molecule; (b) the toroidal model whereby electrostatic interactions between the AMP and the head group of the lipid molecules induces

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significant membrane curvature change along the pore, resulting in a toroidal shape. The carpet model applies to peptides with high positive charges and low hydrophobicity. These remain on the membrane surface and upon reaching a certain concentration, lead to an imbalance of electrical potential (known as depolarization of the membrane) and mechanical force (surface tension), thus disrupting the membrane.

Unsurprisingly, the mechanism of action of AMPs depends on the type of bacteria – (Gram positive or Gram negative) whose differing outer membrane compositions further complicate attempts to elucidate the detailed mechanisms of action. For example, Dings et al. [17] found that cationic and small hydrophobic residues appear crucial for activity against Gram negative bacteria, while larger hydrophobic and cationic residues were active against Gram positive bacteria. This likely arises from the ability of the cationic peptides with small hydrophobic residues to pass through the highly negatively charged outer membrane of the Gram negative bacteria relatively easily, while peptides with higher hydrophobicity are stabilized at the peptidoglycan layer of Gram positive bacteria. Besides the outer membrane, the action mechanism of AMPs also depends on lipid composition of the inner membrane such as phosphatidylglycerol (PG) and phosphatidylethanolamine (PE) lipids, which are the two main components of the inner membrane of most bacteria. It was found that the negatively charged PG lipids are critical for the binding of cationic AMPs [18,19], while the PE lipid, which has an intrinsic negative curvature, is more likely to form membrane pores [20] and has stronger binding free energies than the phosphatidylcholine (PC) lipids [21], the main component of mammalian cells.

We have recently designed a new class of antimicrobial peptides with branched topology [9,22] by cross-linking a C-termini segment of a peptide derived from human beta-defensin [23]. This has led to the development of a series of AMPs in monomeric, dimeric and tetrameric forms which mimic some of the properties of defensins. One of the dimeric peptides (we henceforth refer to it as B2088) is much more active than the corresponding monomer against various bacteria, particularly Gram negative bacteria, with a Minimum Inhibitory Concentration (MIC) that is 20-fold lower [19,22]. We had earlier indicated that B2088 assumed a random structure both in aqueous and lipid environments [19]; in contrast, most AMPs adopt either helical or sheet structures when they bind to membranes. It appears that the lack of stable secondary structure makes B2088 quite flexible and presumably is linked to its broad-spectrum activity.

We had previously demonstrated that B2088 made favorable interactions with LPS molecules, an important component of the outer membrane of Gram negative bacteria [19]. But the molecules still preserved high rotational diffusion rate and some translational mobility, which enabled them to easily pass through the LPS layer and concentrate on the surface of the inner membrane. It appears that when this concentration reaches a critical value, the inner membrane undergoes structural perturbations, resulting in eventual disruption and cell death. The initial membrane interaction is crucial and is assumed to be the rate determining step of the bactericidal process. To probe this process of perturbation in more detail, we present here a study of the interaction of B2088 with model membranes using atomistic molecular dynamics (MD) simulations.

2. Methods

The structure of the model branched peptide B2088 $(RGRKVVRR)_2$-KK is shown in Fig. 1. B2088 was synthesized by cross linking two copies of the C-terminal segment of human beta-defensin at K9, thus resulting in a branched topology with two positively charged N-termini and one negatively charged C-terminus. The protonation state of the charged residues was determined at pH 7. Considering the high pKa values of the cationic residues (10.5 and 12.48 for Lys and Arg respectively), we assume they are protonated during the simulations, which yields a total of 12 positive charges. Of course, it is likely that their pKa values

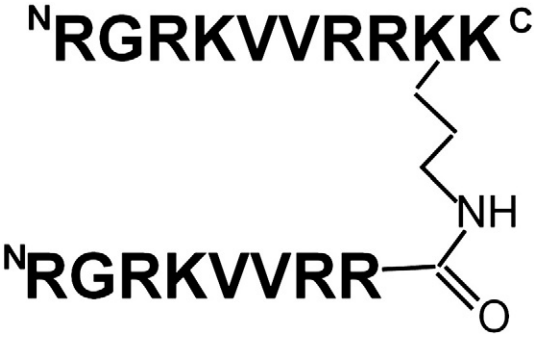

Fig. 1. Chemical structure of B2088.

will undergo shifts in the presence of both the negatively charged membranes and the hydrophobic membranes. In the former, it will be in a direction to keep them positively charged while in the latter they will undergo shifts to lower pKa values, but will still retain them as largely positively charged [24,25]. In order to study the selectivity of B2088, we constructed models of mammalian and bacterial membranes, with each model consisting of 128 lipids. As the mammalian membrane consists mainly of the neutral POPC lipids, we used 128 zitterionic POPC molecules to model the mammalian membrane. In contrast, the bacterial membrane has a more complicated lipid composition, most of which are POPE and POPG lipids. Here we used a mixture of POPE and POPG molecules with a ratio of 3:1 (POPE:POPG) to model the bacterial membrane [26,27]. Although we could use larger membrane patches in our simulations, we believe the finite-size effect is not the critical factor since the electrostatic interactions and the ratio of peptide:lipid is what appears to be the main determining factor (will discuss more later) underlying the peptide–lipid interactions. To construct the model membranes, first we manually put the required number of lipid molecules on grids with a grid spacing of 0.8 nm, resulting in a preassembled bilayer in the xy dimension. Then the preassembled bilayer was solvated with water and neutralized by adding counterions. Subsequently MD simulations were performed for each model membrane until convergence was seen for structural parameters such as area per lipid. Control simulations of each membrane alone were carried out for 200 ns. To examine the concentration dependent effects of B2088, simulations were carried out with varying peptide–lipid ratios: 1:128, 2:128 and 3:128, corresponding to different concentrations of B2088. Initially, one, two and three peptides were placed in random orientations ~5 nm away from the bilayer center with the conformations of the peptides taken from our previous study [28]. Then the system was solvated in a simulation box with roughly 8000 water molecules. Counter ions were added to neutralize the system. As cationic antimicrobial peptides display reduced activity at high salt concentrations, to examine the interaction of B2088 with the bacterial membrane at levels at which optimal activity was observed, we simulate the dynamics under low salt concentrations (0.02–0.1 mM), as used in the experiments. Further, to sample the phase space more efficiently, three copies of each simulation were run using different initial orientations of B2088. To keep the membrane in the fluid phase and to accelerate the equilibration, we performed all the simulations at 323 K, which we believe is higher than the phase transition temperatures of POPE/POPG and POPC bilayers, as has been used in a number of simulation studies [29–32].

We have previously found that the CHARMM27 force field without cMAP correction appears to be best parameterized to yield conformations of B2088 that were most consistent with experimental data [28]. The recently released CHARMM36 force field was used to model the lipids as it was shown to predict the correct area per lipid and acyl chain order parameters for a number of lipids without applying surface tension [33]. The TIP3P model was used for water. The covalent bonds between hydrogen atoms and any heavy atoms were constrained using the LINCS algorithm [34], which enabled a time step of 2 fs to be