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 guanidinium 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.

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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
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 phosphatidyglycerol (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 (RGRKVVRRRK)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 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 zwitterionic 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 POPE 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 to 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 POPE 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
used in all the simulations. A cutoff distance of 1.2 nm was used for both the LJ and real-space electrostatic interactions, and the particle-mesh Ewald algorithm was employed to calculate the long-range electrostatic interactions in reciprocal space [35]. The Nose–Hoover coupling method was used to maintain the target temperatures at 323 K and the semi-isotropic Parrinello–Rahman method was used to maintain the pressure at 1 atm in the NPT ensemble [36]. All the simulations were run for at least 200 ns using the GROMACS package 4.5 [37]. Membrane properties such as area per lipid, order parameter of the acyl chains, the density profile of different groups, the peptide-membrane distance, and the number of hydrogen bonds between different groups were calculated using tools in the GROMACS package. The number of water translocation events across the membrane was calculated using the g_flux script developed by Beckstein et al. [38]. For the surface potential of the model membranes, we used the APBS plugin of PYMOL [39,40].

3. Results and discussions

3.1. Peptide B2088 interacts more favorably with a model bacterial membrane

Our previous studies have shown that B2088 has excellent killing ability towards bacterial cell, while maintaining low toxicity against mammalian cells. To understand this selectivity, we performed MD simulations using different concentrations of B2088 in the presence of mammalian and bacterial membranes, respectively. Figs. 2 and S1 shows that when there is a single molecule of B2088, it rapidly adsorbs onto the bacterial membrane (within 5 ns), driven by strong long-range electrostatic attractions between the positively charged peptide and the negatively charged membrane. Upon adsorption, the affinity of B2088 for the bacterial membrane is further enhanced as a result of the formation of many hydrogen bonds between B2088 and lipid molecules (discussed later). In contrast, in the presence of the mammalian membrane, B2088 oscillates in its diffusion and adsorbs onto the membrane after a much longer time (more than 120 ns), indicating a very weak affinity. This is not surprising since the mammalian membrane is neutral and the long-range electrostatic interactions will be much weaker compared to those in the bacterial membrane. However, once adsorbed on the mammalian membrane, B2088 remained at the membrane–water interface for the rest of the simulation. Although the mammalian membrane is neutral, its constituent, zwitterionic POPC contains negatively charged PO4 groups that form hydrogen bonds with the basic side chains (e.g., Arg and Lys) of B2088. To further understand the importance of electrostatic interactions, we decomposed the non-bonded interactions of B2088 with the two model membranes into LJ and real space Coulomb interactions (Fig. S2). It is clear that the electrostatic interactions of B2088 with the bacterial membrane dropped to around ~1500 kJ/mol within 10 ns, at which point the peptide makes contact with the membrane surface prior to forming short-range hydrogen bonds with the lipids. This leads to a further stabilization of the electrostatic interactions between B2088 and the bacterial membrane that finally stabilizes around ~2000 kJ/mol. In contrast, the electrostatic interactions between B2088 and the mammalian membrane were much weaker (~1200 kJ/mol). However, the vdW interactions of B2088 with the two membranes were similar (both fluctuate around ~200 to ~300 kJ/mol), suggesting that electrostatics were clearly the likely discriminating factor in the selectivity of the interactions of B2088 for the bacterial over the mammalian cells. This in turn leads to changes in packing between the atoms. The density distributions of peptide atoms with respect to the phosphate atoms (Figs. 3 and S2) are more narrowly distributed and closer to the phosphate peaks of the bacterial membrane, resulting from B2088 becoming more tightly embedded in the bacterial membrane.

3.2. The interfacial activity determines the selectivity of B2088

To study the effect of peptide concentration on the membranes, we next performed simulations with various peptide:lipid ratios in the case of the two model membranes. As B2088 is highly cationic, a strong repulsion between B2088 molecules is expected. Once a B2088 molecule adsors on the membrane surface, it is difficult for another B2088 molecule to get adsorbed nearby. This leads to a technical difficulty which was that upon the adsorption of some B2088 molecules on the upper leaflet of the membrane, the repulsion causes the other molecules of B2088 to move away from the surface and re-enter from the bottom of the simulation box (this arises due to the periodic boundary conditions that are used to simulate such systems). They subsequently get adsorbed on the lower leaflet of the membrane, which corresponds to the intracellular surface of the membrane. To overcome this problem, we first applied a weak pulling force with a harmonic potential of 50 kJ/mol/nm² between the center of mass of each B2088 molecule and the membrane. This resulted in all the B2088 molecules being fully adsorbed on the membrane. The pulling force was then removed and simulations continued for 200 ns, which has been used to study the cell penetrating peptides [41]. We found that as a result of the extensive hydrogen bonds and strong electrostatic interactions that clearly stabilize the system against the intermolecular repulsions, up to 3 B2088 molecules could remain stably bound to the bacterial membrane (Fig. S3). In contrast, in the mammalian membrane, the lower density of hydrogen bonds and weaker electrostatics were insufficient to overcome the intermolecular repulsions and even two B2088 molecules were unable to stay together on the membrane surface (Fig. S4). The electrostatic surface potential (Fig. 4) of the bacterial membrane is very negative while it is neutral for the mammalian membrane. It was

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**Fig. 2.** The z component of the distance between the center of mass of B2088 and the bilayer center, showing the difference in the adsorption kinetics on bacterial and mammalian membrane. The other two copies of simulations using different initial orientation of B2088 show similar trend.

**Fig. 3.** Distributions of peptide atoms and phosphate atoms of the two leaflets of both bacterial and mammalian membranes.
clear that even after the adsorption of one or two B2088 molecules on the bacterial membrane, patches with negative potential remained on the surface and it was only after the addition of the 3rd molecule of B2088 that the surface potential was saturated, becoming positive. In contrast, one B2088 was sufficient to saturate the surface potential of the mammalian membrane. This begins to explain the observation of a low surface concentration of B2088 on the mammalian membrane and hence the selectivity for the bacterial membrane, in agreement with the interfacial activity model [7]. We next analyzed the effect of changing the surface concentration of B2088 on the structural properties of the bacterial membrane.

3.3. The bactericidal activity of B2088 is concentration dependent

An important structural parameter of membranes is the area per lipid (APL). Fig. 5 shows the APL of the model bacterial membrane in the presence of different numbers of B2088 molecules (the APL of all the 3 simulations are shown in Fig. S5). For the bacterial membrane

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**Fig. 4.** Surface potential of pure mammalian (a) and pure bacterial (b) membranes. (c) is for the case of one B2088 on mammalian membrane. (d)–(f) are for bacterial membrane with one (d), two (e) and three (f) B2088 molecules. Blue patches indicate positive potentials, while red ones indicate negative potentials.
higher B2088 concentration induces large width of each phosphate peak became broader, suggesting that the distribution of water molecules around lets. Also shown in Fig. S6 is the water density pro

brane thinning effect, which is consistent with the results for other membranes. In our simulations, at high B2088 concentrations (e.g., using three B2088 molecules), significant structural perturbations of the bacterial membrane occur, in agreement with the interfacial activity model [7]. In contrast, the simulations suggest that B2088 can only assemble at very low concentrations on mammalian membranes.

Deuterium order parameters of the lipid tails (Fig. 7) decreased dramatically only in the presence of three B2088 molecules. Besides the decreases in the order parameters of atom 9, which arises from the presence of the double bond in the sn2 chain, there is a slight decrease in the order parameter of atom 6 in all simulations. This is different from that seen in other studies [27,47] and may result from finite size effects [48], the elevated temperature or the different force field used in the simulations [49]. The less ordered lipid tail results from the membrane expansion in the xy plane and thinning in the z dimension. Increased disorder of the lipid tails is expected to affect the fluidity of the lipid molecules that in turn is likely to influence the physiological functions of the bacterial cells [50].

It should be noted that only 128 lipid molecules were used in each simulation due to the limited computational resources. The lack of long range undulations may lead to finite size effect in the estimation of the membrane properties. Several studies have shown that finite size effects can arise from inappropriate heat coupling [51], the lipid:water ratio [52], or the treatment of electrostatic interactions [53,54]. More recently, Carola and Knecht found that the finite size effect leads to underestimation of the binding free energies of Nk-2 to both POPE and POPC bilayer, with the magnitude of deviation being larger for POPE than POPC, resulting in an overestimation of 2.6 kcal/mol of transfer free energies from POPC bilayer to POPE bilayer [21]. To solve this, they proposed a new way of correcting the free energy. For our simulations, it is likely that the binding of B2088 to the membrane may be underestimated for both the bacterial and the mammalian membranes. However, since in our simulations, we use a model membrane containing a mixture of zwitterionic POPE and anionic POPG lipids instead of the pure POPE bilayer, the correction term for the binding free energy of B2088 is expected to be smaller as the binding of B2088 to the bacterial membrane (mixed POPE and POPG lipids) is mainly driven by electrostatic interactions. Therefore we believe that the magnitude of the correction term only affect our results quantitatively. Indeed, a number of simulation studies of the antimicrobial peptides have used 128 lipids with various numbers of peptides [15,55–58].

In summary, the above results show that at low surface concentrations, B2088 has a minor effect on the structural properties of the bacterial membrane. However, at high concentrations (e.g., using three B2088 molecules with 128 lipid molecules), significant structural perturbations of the bacterial membrane occur, in agreement with the interfacial activity model [7]. In contrast, the simulations suggest that B2088 can only assemble at very low concentrations on mammalian membranes.

3.4. B2088 leads to increases in membrane permeability

At high B2088 concentrations, we also observed several events of water translocation across the bacterial membrane. In the absence of B2088, this was a rare event because of the high free energy barrier of transferring polar water molecules across the hydrophobic environment of the membrane [59]. A similar observation was reported for the antimicrobial peptide Maculatin 1.1 whose presence was found to be associated with a large number of water translocations, based on a coarse-grained model [60]. In our simulations, at high B2088 concentrations (e.g., peptide:lipid = 3:128), the membrane undergoes large fluctuations and deformations, which leads to many water-insertion defects in the hydrophobic region of the membrane. Although most of the defects are transient and have a short lifetime (of ns), some of the water molecules further penetrate into the hydrophobic region and eventually

![Fig. 5. The area per lipid (APL) of the model bacterial membrane in the presence of different number of B2088 molecules.](Image)

![Fig. 6. Distribution of the minimum distance between the phosphate atoms of the two leaflets. The data are averaged over 3 copies of simulations.](Image)
Fig. 7. Deuterium order parameters of the two acyl chains (sn1 and sn2) of POPE and POPG. The data are averaged over 3 copies of simulations.

Fig. 8. Snapshots showing the water defects and water translocations across the membrane.
translocate across the membrane (Fig. 8). It appears that once a single water molecule enters into the hydrophobic core, it diffuses rapidly and randomly in the hydrophobic region as it would do in the gaseous state. In some cases they diffuse back to the head group region of the same leaflet, while in other cases they can cross the bilayer center and reach the opposite leaflet. Previous studies have shown that the free energy barrier of transferring water molecules across the membrane arises from the dense packing of the acyl hydrocarbon chains in the lipid tail region [61]. Our current results show that the high surface concentration of B2088 leads to membrane expansion, thus reducing this packing density. This in turn appears to generate large free volumes in the hydrophobic region. At the same time, the lipid acyl chains undergo significant disorder. Together, these are expected to lead to an increase in the number of cavities in the lipid tail region, and are possibly responsible for the increased water permeability. To carry out a cavity search in the hydrophobic region of the membrane the hydrophobic region was defined as the distance between the average positions of the phosphate atoms of the two leaflets in the z direction. Subsequently the hydrophobic region was divided into a number of grids with grid size 0.05 nm. Probes of different diameters were placed at every grid point to check the overlap with the lipid atoms. The cavity probability is calculated by dividing the total number of grids by the number of non-overlapping grid points (Fig. 9). As expected, the cavity probability increases significantly with decreasing probe size. It can also be seen that for all probe sizes used, the higher surface concentration of B2088 (peptide:lipid ratio of 3:128) is associated with increased membrane permeability. The number of water translocations across the membrane during the last 100 ns of the simulations in the presence of 0, 1, 2 and 3 molecules of B2088 respectively was 5, 9, 9 and 37. Note that while this enhanced permeability of water is obtained at 323 K, at the physiological temperature of 310 K, the concentration dependence of membrane permeability still holds true; the magnitude of the cavity probability and the number of water translocation will likely be slightly reduced. In summary, it appears that beyond a certain concentration of B2088, the bacterial membrane undergoes significant distortions that can enable enhanced permeability of molecules such as water.

3.5. Hydrogen bonding analysis: importance of Arg

As mentioned in Section 3.1, short-range hydrogen bonds are important determinants of the interaction of B2088 with the bacterial and mammalian membranes. The average number of hydrogen bonds (Table 1) between the lipid molecules in the bacterial membrane was 150 at low concentrations of B2088, but drops by 10% when the 3rd molecule of B2088 was present; this is unsurprising since the membrane undergoes expansion, a feature that is likely to change the membrane elasticity. Each B2088 molecule forms about 25 hydrogen bonds with the bacterial membrane and only 15 hydrogen bonds with the mammalian membrane, mostly involving the side chains of basic residues of B2088. This was expected since B2088 has 8 Arg and 3 Lys side chains that can form hydrogen bonds with lipid molecules, while the backbone atoms of B2088 are mostly buried. Further analysis revealed that it was the PO4 groups of the lipids that form the majority of the hydrogen bonds with B2088. Arg side chains form more hydrogen bonds with PO4 group than Lys side chains do. For example, in the case of three B2088 molecules, the number of hydrogen bonds between Arg side chains and the PO4 groups is 51, accounting for 68% of the total number of hydrogen bonds between B2088 and the lipid molecules. Visual inspection of the simulations suggested that the guanidinium group of the Arg side chains forms stable bidentate hydrogen bonds with PO4 groups, which are not only geometrically favorable, but also energetically more stable (Fig. 10). Such bidentate hydrogen bonds between Arg side chains and PO4 groups have been reported in other computational and experimental studies [62,63]. This feature is believed to play an important role in inducing negative curvature in anionic bacterial membrane [64]. This powerful hydrogen bonding ability of Arg is coupled to the planar structure of the guanidinium group whose hydrophobic character enables it to easily penetrate into the membrane [65]. Together, these unique properties endow Arg residues with a high affinity for lipid molecules, and are hypothesized to play an important role in the membrane perturbations induced by B2088.

4. Implications for the mechanism of action

Although we did not observe complete membrane disruption by B2088 in our simulations (this process presumably occurs at much longer time scales), we develop some insights into the early stage of the action mechanism of B2088. Several studies have underscored the importance of electrostatic interactions in mediating the adsorption of AMPs onto the surface of bacterial membranes [66–68]. We find that in addition, hydrogen bonding also contributes significantly to interactions between B2088 and the bacterial membrane. The reduced toxicity of B2088 likely results from its low surface concentration on the mammalian membrane, which appears to arise from reduced electrostatic attractions and hydrogen bonds between B2088 and the mammalian membrane, i.e., lower interfacial activity [7]. In addition, unlike mammalian cells, the highly negatively charged outer membrane of most bacterial cells (particularly Gram negative bacteria) serves to concentrate molecules such as B2088 around the surface of the inner membrane, thus further contributing to the selectivity.

When B2088 reaches a high surface concentration on the bacterial membrane, it appears to induce large structural perturbations, such as increased area per lipid, enhanced fluctuations and reduced order parameters. All these structural parameters showed concentration dependence. Our results suggest that it is only at high concentrations (peptide: lipid ratio of 3:128), that bacterial membrane perturbation is significant, leading to reduced stability.

The structural perturbations of bacterial membranes observed at high surface concentration of B2088 are associated with the formation of water defects and subsequent water translocations across the membrane, suggesting that the membrane becomes leaky. It would be interesting to explore this process for larger membrane patches and at longer time scales to see if larger defects form that in turn would facilitate the permeation of larger molecules including water clusters. This begins to provide some insight into the molecular origins of the experimentally observed minimum inhibition concentrations needed to kill bacteria.

What lessons can be learnt for further peptide design? The above results indicate that B2088 prefers to stay on the membrane surface, perturbing the membrane–water interface, suggesting a carpet-like mechanism of action. Long-range electrostatic interactions drive the

**Fig. 9.** Cavity probability in the lipid tail region of the bacterial membrane. The data are averaged over 3 copies of simulations.
highly positively charged B2088 molecules to accumulate onto the negatively charged surface of the bacterial membrane. The flexibility of B2088 hence enables it to maximize its interactions with the lipid molecules, notably through the large number of hydrogen bonds between the side chains of basic residues of B2088 (e.g., Arg and Lys) and the PO4 groups of lipid molecules. The large contact area and strong interactions between B2088 and the membrane surface further appears to induce membrane deformation. This eventually results in a significant change in membrane properties such as a less ordered liquid phase associated with increased permeability. The membrane thus becomes leaky and perhaps enables intracellular molecules to permeate through the membrane. It is clear that a combination of positive charges and flexibility is required to achieve maximum membrane perturbations; Arg side chains seem to be appropriate for this by combining cationicity, large number of hydrogen bonding sites, and planar hydrophobic moieties, that enable insertion into and perturbation of the membrane surface.

5. Summary

We have examined the interaction of a novel, branched antimicrobial peptide B2088 with model bacterial and mammalian membranes in order to gain insights into its mechanism of action, notably its rapid action against bacterial and reduced cytotoxicity against mammalian cells. We found that the high content of basic residues in B2088 leads to strong electrostatic interactions and large number of hydrogen bonds with the anionic surface of model bacterial membranes. In contrast, the lower density of charged groups on mammalian membranes leads to weak interactions and smaller number of hydrogen bonds. As a result, B2088 can achieve a much higher surface activity on the bacterial membrane than on the mammalian membrane, accounting for its high selectivity. The larger number of hydrogen bonding interactions arises primarily from the bidentate hydrogen bonds that are formed between the guanidinium group of Arg and the PO4 groups of the lipid molecules. Increased concentrations of B2088 uniquely perturb the membrane by perturbing the area per lipid, density profiles and order parameters of lipid tails. Structural perturbations of the membranes are associated with enhanced permeability, as revealed by the large number of water translocations across the membrane.

* Movies of B2088 adsorption on models of bacterial and mammalian membranes and configurations of model bacterial and mammalian membranes consisting of 128 CHARMM lipid molecules can be found at: http://web.bii.a-star.edu.sg/~lijg/downloads.html

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Appendix A. Supplementary data

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