How Can a β-sheet Peptide Be Both a Potent Antimicrobial and Harmfully Toxic? Molecular Dynamics Simulations of Protegrin-1 in Micelles

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Abstract: In this work, the naturally occurring β-hairpin antimicrobial peptide protegrin-1 (PG-1) is studied by molecular dynamics simulation in all-atom sodium dodecyl sulfate and dodecylphosphocholine micelles. These simulations provide a high-resolution picture of the interactions between the peptide and simple models of bacterial and mammalian membranes. Both micelles show significant disruption, as is expected for a peptide that is both active against bacteria and toxic to host cells. There is, however, clear differentiation between the behavior in SDS versus DPC, which suggests different mechanisms of interaction for PG-1 with mammalian and bacterial membranes. Specifically, the equilibrium orientation of the peptide relative to SDS is a mirror image of its position relative to DPC. In both systems, the arginine residues of PG-1 strongly interact with the head groups of the micelles. In DPC, the peptide prefers a location closer to the core of the micelle with Phe12, Val14, and Val16 imbedded in the core and the other side of the hairpin, which includes Leu5 and Tyr7, located closer to the surface of the micelle. In SDS, the peptide prefers a location at the micelle–water interface. The peptide position is reversed, with Leu5 and Cys6 imbedded furthest in the micelle core and Phe12, Val14, and Val16 on the surface of the micelle. We discuss the implications of these results with respect to activity and toxicity.


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INTRODUCTION

Resistance to established antibiotics is a growing problem that has stimulated interest in the development of novel antimicrobial agents. Protegrins are a family of five potent, naturally occurring, cationic antimicrobial peptides (AMPs) that were originally purified from porcine tissue. They combine features of defensins and tachyplesins, having two disulfide bonds and a β-sheet structure, and are small, only 16 to 18 residues in length. Protegrin-1 [PG-1, R G G R L C Y C R R F C V C V G R] can launch a rapid response to infection by diverse bacterial species including Escherichia coli, Candida albicans, and Listeria monocytogenes. For PG-1, the minimum inhibitory concentrations (MIC) against various...
species were determined as summarized in reference 5. Because of its activity profile, and because microorganisms cannot easily develop resistance to this as well as other antimicrobial peptides, protegrins have potential for pharmaceutical use.\textsuperscript{4,6} Specifically, the development of a protegrin-derived peptide is desired for use in a topical agent to prevent transmission of sexually transmitted diseases such as Neisseria gonorrhoeae,\textsuperscript{3} Chlamydia trachomatis,\textsuperscript{7} and human immunodeficiency virus type 1 virions.\textsuperscript{3,8} Unfortunately, protegrin-1 is also toxic. PG-1 lyses more than 50% of red blood cells at 80 \(\mu\)g/mL of peptide and requires a concentration of 25–50 \(\mu\)g/mL for EC\textsubscript{50}, making it both hemolytic and highly toxic. The low values for MICs [0.9 \(\mu\)g/mL against E. coli, P. aeruginosa, and L. monocytogenes; 1.2 \(\mu\)g/mL against N. gonorrhoeae F-62, 1.7 against N. gonorrhoeae FA-16, and 5.3 against C. albicans] show the great activity of this peptide against many bacterial species; however, the high values for hemolysis and cytotoxicity mean that the peptide kills a significant number of human cells. This hinders efforts to use naturally occurring PG-1 for therapeutic purposes and generates the need to understand the sequence or structural components of this peptide and other AMPs that are responsible for activity and toxicity.

For antimicrobial peptides, it is widely accepted that the main target is the lipid bilayer of the bacterial cell membrane itself, instead of specific protein receptors within the cell membrane.\textsuperscript{1,6,9} It is supposed that the differences in composition between bacterial and mammalian cell membranes provide a way for AMPs to distinguish between mammalian and bacterial cell membranes.\textsuperscript{10–12} However, numerous AMPs, like protegrin, also disrupt mammalian cell membranes. Any attempt to design a peptide for therapeutic use would then be aided by a clear understanding of the structural elements that are responsible for the interaction of the peptide with both mammalian and bacterial cell membranes. A clear picture would then help in engineering a new sequence to retain the antimicrobial characteristics while eliminating the toxic characteristics of the peptide, assuming that they are distinct and separable in the structure.

The lipid composition of the surface of a prokaryotic cell is quite different from that of a eukaryotic cell. Mammalian cells are comprised mainly of phosphatidylcholine (PC), sphingomyelin, and cholesterol, all of which are neutrally charged at physiological pH.\textsuperscript{13} On the other hand, bacterial membranes include substantial amounts of negatively charged lipids such as phosphatidylglycerol and cardiolipin.\textsuperscript{9} To elucidate how antimicrobial peptides, protegrin-1 in particular, interact with the constitutive components of both bacterial and mammalian cell membranes, we simulate the interactions of PG-1 with a dodecylphosphocholine (DPC) micelle and a sodium dodecylsulfate (SDS) micelle, a mammalian and a bacterial membrane mimic, respectively. Micelles possess a well-defined hydrophobic core and a flexible, hydrophilic interface like lipid bilayers. Indeed, micelles are commonly used in place of monolayers or bilayers of phospholipids in methods such as NMR spectroscopy.\textsuperscript{14–16} DPC micelles are good models of eukaryotic cell membranes, which are generally rich in zwitterionic phospholipids. SDS is a suitable mimic for the negatively charged molecules found in bacterial membranes.\textsuperscript{13} Although there is no direct correlation between the SDS head group and the head groups commonly found on the phospholipids composing a bacterial membrane (as there is between DPC and PC phospholipids), the SDS micelle is generally agreed to be a good model bacterial membrane because it possesses an anionic exterior and a hydrophobic interior.\textsuperscript{17–21} The differences in charge and composition between the SDS micelle and the DPC micelle provide a basis for the study of the activity and toxicity of antimicrobial peptides.

Both SDS and DPC micelles have been successfully modeled in molecular dynamics simulations in CHARMM\textsuperscript{22–25} due to their fast (much faster than lipid bilayers) time scales of lipid motion. Their relaxation times have been shown, through experiment and simulation, to be on the order of 500 to 1000 ps,\textsuperscript{24–26} compared to the tens of nanoseconds required for lipid bilayers.\textsuperscript{27} The micelle system also contains approximately half of the number of atoms of a typical 128-molecule lipid bilayer–peptide–water simulation. This allows a much longer simulation time, permitting for monitoring of biological phenomena of longer time scales using the same computational resources, rendering micelles an attractive alternative to lipid bilayers.

Molecular dynamics simulations in micelles have been shown to be particularly successful in providing an atomistic picture of the interaction of peptides with membrane mimics. Previous studies of peptides with membrane mimics such as SDS or DPC micelles have demonstrated that such simulations can indeed provide a detailed picture of the behavior of peptides in heterogeneous environments.\textsuperscript{28,29} Specifically, Gao and Wong\textsuperscript{30} has studied the interactions of adrenocorticotropic hormone (ACTH) in solvated DPC micelles and succeeded in determining the equilibrium conformation. Additionally, for ACTH, Wymore and Wong\textsuperscript{31} demonstrated through MD simulations that the membrane can induce secondary structure changes in the peptide that may facilitate peptide–receptor recognition processes. Nisin was studied by van den Hooven and coworkers.\textsuperscript{32}
in both DPC and SDS micelles to determine its structure–function relationship. In 2000, Rozek and coworkers performed a similar study with the bovine antimicrobial peptide indolicidin in both DPC and SDS micelles.

Also, we have previously studied the HIV-1 fusion peptide FP-1 in an SDS micelle, and the antimicrobial ovispirin and its derivatives in both SDS and DPC. We detail here the simulation method used, present the results, and discuss the hypotheses we can generate about the activity and toxicity of protegrin-1.

**METHODS**

PG-1 in SDS

The starting coordinates of the SDS micelle–water complex were obtained from simulations carried out by MacKerell. The methodology largely follows that outlined in our previous work.

Briefly, each micelle consists of 60 SDS molecules and is solvated with 4375 water molecules. The simulation box has an initial size of 54.15 Å. Water is modeled using the TIP3P potential. For SDS, 60 sodium counterions are added along with 0.15 M NaCl electrolyte ions, which are randomly distributed in the aqueous phase. The initial structure of PG-1 was obtained from the PDB (www.rcsb.org, PDB ID: 1PG1). The CHARMM program, its force field, and parameters are described in detail by both Brooks et al. and MacKerell et al.

Solid-state NMR experiments of PG-1 in DLPC bilayers suggest that the peptide orients itself inside the bilayer with the C- and N-termini interacting with the head groups of one layer. The β-turn interacts with the opposite layer’s headgroups, with the β-strand axis tilted by 55° from the bilayer normal to allow the central region of the peptide to interact with the hydrophobic core. Using this configuration as the starting point, we placed the peptide in the center of the micelle core with the micelle center of mass overlapping with the center of mass of the peptide, as shown in Figure 1. Owing to the spherical symmetry of the micelle, the orientation of the peptide is unimportant. To eliminate any initial conformational bias, simulations have also been run with the peptide starting outside of the micelle, within 5 Å of the outer surface atoms of micelle molecules. As we discuss under Results, the peptides stabilize themselves in the same equilibrium orientation in both simulations.

To remove initial bad contacts between the peptide and the core and to prevent penetration of water during equilibration, the peptide and bulk water were initially kept under weak harmonic constraints with spring constants of 10 and 5 kcal/mol Å, respectively. The constraints were gradually removed in 20,000 steps of minimization (steepest descent method). The entire system was then minimized for 20,000 more steps without any constraints. Thereafter, the system, consisting of approximately 16,000 atoms, was gradually heated to 303.15 K. After 500 ps of equilibration, the entire assembly was subjected to NPT dynamics at a pressure of 1 atm and a temperature of 303.15 K. The constant pressure–temperature module of CHARMM was used for the simulations with a leap-frog integrator (2 fs time step). The temperature was set at 303.15 K using the Hoover temperature control. All the components of the piston mass array were set to 500 amu for the extended system pressure algorithm. The electrostatic interactions are simulated using the particle mesh Ewald (PME) summation without truncation and a real space Gaussian width of 0.25 Å⁻¹, a β-spline order of 4, and a FFT grid of about one point per Angstrom. All simulations were carried out using CHARMM version c29b2 with the param22 parameter set. The simulation in SDS was carried out for 13 ns; to our knowledge, the longest simulation to date in SDS was 5 ns.
PG-1 in DPC

The protocol followed for PG-1 in DPC is the same as that used for PG-1 in SDS. The initial coordinates for the DPC micelle were obtained from Wong and Kamath. The micelle was initially constructed using CHARMM version 24b2 with the CHARMM all22 force field for the lipids. The parameters from the dodecyl chain of dodecylsulfate were combined with those of the phosphocholine head group to create a dodecylphosphocholine molecule. The DPC micelle was built using the same procedure as the SDS micelle, using 60 DPC molecules and aligning each along vectors extending from the center of a C60 buckminsterfullerene molecule through the individual carbons of the C60 molecule. The C12 methyl carbons were initially placed 3 Å from the center of the fullerene molecule with the DPC chain extending outward. As with the simulation in SDS, the peptide was placed in the core of the micelle with the center of mass of the peptide overlapping the center of mass of the micelle (Figure 2). To this, 4377 water molecules and NaCl electrolyte to a concentration of 0.15 mM were added into the 54.15 Å simulation box. The system was then minimized and the NPT simulation at 1 atm and 303.15 K was performed as in the SDS system.

RESULTS

All properties were calculated from the atom positions and velocities stored in CHARMM trajectory files. We can assume that equilibrium has been reached for at least the last 3 ns of the simulation of the SDS system and the last 5 ns of the DPC system, since the peptide does not change location or orientation with respect to the center of mass of the micelle. Time-averaged properties were calculated from 10 to 13 ns for the SDS system and from 35 to 40 ns for the DPC system.

Relative Peptide–Micelle Positions

The final locations of PG-1 with respect to the micelles are shown in Figure 3. In SDS, the peptide moves rapidly from the center of the micelle to the surface. In DPC, however, the peptide remains inside of the micelle for a significantly longer period of time.

The distance between the center of mass of the micelle and the center of mass of the peptide was calculated in all simulated systems as a means of identifying the final position of the peptide and determining when equilibrium was reached. In SDS, starting both from inside of the micelle and from outside of the micelle, in two independent simulations, the peptide reaches the same location with respect to the micelle center in approximately 8 ns. It is particularly encouraging that, in SDS, the peptide’s position and orientation equilibrate to the same stable conformation for two independent simulations, starting from inside the micelle core and starting from outside the micelle surface. This is an important result because it demonstrates that all computed properties are not dependent on the initial conformation.

This significant result is also obtained with the two simulations of PG-1 in DPC. Interestingly, the movement of PG-1 in DPC is much slower, requiring

FIGURE 2 Initial views of the PG-1 in DPC system (in stereo). DPC is shown in green and PG-1 is in orange. Water is in cyan. The initial position of the peptide has its center of mass overlapping the center of mass of the micelle.
nearly 35 ns for the peptide to move to its equilibrium position when starting from inside the micelle.

This position is approximately the same distance from the micelle center as in SDS, but due to the difference in size between the two types of micelles, PG-1 is more imbedded into DPC than it is in SDS (Figure 4).

We also calculated the distance from the center of the micelle for each residue. In Figure 5 we show the final structure of the peptide colored by residue according to the residue location with respect to the micelle. The first 10 residues in SDS (Figure 5A) are located in the micelle core (red), and the remainder of the peptide is located at the interface (violet) and in the bulk water (blue). The opposite occurs for PG-1 in DPC (Figure 5B), with the residues around Phe^{12} located farthest inside the micelle and the residues near Leu^{5} located in the bulk water. A more in depth discussion of the location of specific residues will follow in the remaining sections (Electron Density Profiles and Radial Distribution Functions, and Hydration Numbers).

**FIGURE 3** (A) Snapshots of PG-1 in SDS micelle. The peptide started from the initial position shown in Figure 1 and moves to the interface in 1 ns, the peptide has already moved from the micelle core to the surface. The peptide is shown in orange with Phe^{12} in blue and Leu^{5} in red in a gray SDS micelle. (B) Snapshots of PG-1 in DPC micelle. The peptide progresses much slower than in SDS and does not move out of the micelle core until after 20 ns. The peptide is in orange here, and the micelle in green. Phe^{12} is shown in blue and Leu^{5} is in red. The differences in interaction for PG-1 with SDS and DPC are apparent at the end of the simulations, as Phe^{12} interacts with DPC but not SDS and Leu^{5} interacts with SDS but not DPC.

**Peptide Influence on Micelle Shape**

The effect of the peptide on each micelle can be appreciated by comparing properties of the micelle in the presence of the peptide to properties computed for the pure micelle. First, the radius of gyration was calculated for both micelles. The average values for SDS and DPC in our simulations were 16.0 and 17.2 Å, respectively. Published values for the pure micelles are 16.0223 and 17.4 Å, respectively. Although there is little variation in the radius of gyration, ratios of the moments of inertia were calculated to determine the sphericity of the micelle. These values show significant deviation from the pure micelle. Three moments of inertia ($I_x$, $I_y$, and $I_z$) were calculated along arbitrarily determined orthogonal vectors. $R_1$, is the ratio of $I_y$ and $I_x$, and $R_2$ is the ratio of $I_z$ to $I_x$. For a perfect sphere, both of these ratios would be equal to one. MacKerell^{23} reported values for a pure SDS micelle of 1.04:1.03:1. The results from our simulation give an average ratio of 1.50:1.34:1. This difference of over 30% indicates a significant disruption of the micelle.
For DPC, Wong reports ratios of 1.34:1.22:1. Our simulations give an average ratio of 1.40:1.28:1, significantly less disruption compared to the SDS micelle.

Dihedral Angles

The dihedral angles show that PG-1 is clearly a \( \beta \)-hairpin in both DPC and SDS. Typical values for \( \beta \)-sheet dihedral angles are \( \phi = -120^\circ \) and \( \psi = 100^\circ \). As shown in Table I, in SDS, residues 4 through 8 and 11 through 18 are \( \beta \)-sheet, with a turn at residues Arg\(^{10}\) and Arg\(^{11}\). In DPC, the peptide is \( \beta \)-sheet for residues 4 through 9 and 12 through 17. The turn is centered at residues 10 and 11. Residues 1–3 and 18 are random conformations in both systems. The highest degree of flexibility is found at the C- and N-termini in both systems, particularly at residues 1 through 3 and Val\(^{16}\) and Gly\(^{17}\). The rest of the structure is constrained by two disulfide bonds.

Interaction Energies

To follow the evolution of the interactions between the peptide and micelle, the electrostatic and van der Waals interaction energies were calculated for the peptide and each micelle. Averaged over the last 5 ns of simulation, the values for electrostatic interaction energy are \(-353 \pm 62 \text{ kcal/mol}\) and \(-317 \pm 60 \text{ kcal/mol}\), for PG-1 with the SDS and DPC micelles, respectively. The values for the electrostatic interactions are very similar, due to the interactions between the arginine residues and the micelle head groups. The average total van der Waals interactions for the peptide and SDS micelle are \(-98 \pm 9 \text{ kcal/mol}\) and \(-67 \pm 14 \text{ kcal/mol}\) with the DPC micelle. The electrostatic interactions are significantly stronger than the van der Waals interactions due to the presence of 6 arginine residues on PG-1 that interact with the negative charges on the SDS and DPC head groups.

It is interesting to consider the van der Waals interactions between the hydrophobic residues of PG-1 and the micelles. In Figure 6, the interactions between four of the main hydrophobic residues and the micelles are shown. Most notable are the differences in interactions between Leu\(^5\) and the micelles and...
Phe\textsuperscript{12}. Leu\textsuperscript{5} shows more attraction with the SDS micelle than the DPC micelle, while Phe\textsuperscript{12} shows more interaction with the DPC micelle than the SDS micelle. There are variations for the valine residues, though they show approximately the same magnitudes of interaction.

Electron Density Profiles

Electron density profiles were calculated for each major functional group in the system. To obtain a clear picture of how these groups interact at equilibrium, we studied the methyl groups, the carbon chains, and the sulfate head groups of SDS; the methyl groups, carbon chains, phosphate, and choline head groups of DPC; the water, sodium, and chlorine; and each residue of the peptide. Electron density profiles were computed based on the radial distance from the center of mass of the micelle outward and averaged over the last 500 ps of simulation (Figure 7).

The electron density profiles suggest different modes of interaction between PG-1 and the two micelles. Although the peptide settles itself at the interface in both systems, its orientation in one micelle is the opposite of its position in the other.

In DPC, Phe\textsuperscript{12} is located farthest inside the micelle, with the residues nearest to it also being clearly imbedded in the micelle interior including Val\textsuperscript{14}, as can be seen in Figure 7A. Farthest from the micelle interior and located on the micelle surface are the arginine residues at the beginning of the turn, Arg\textsuperscript{9} and Arg\textsuperscript{10} (Figure 7E). Tyr\textsuperscript{7} and Leu\textsuperscript{5} are also located close to the micelle surface even though they are hydrophobic.

In SDS, Leu\textsuperscript{5}, Cys\textsuperscript{6}, and Cys\textsuperscript{8} are clearly located in the micelle core (Figure 7B and D). Phe\textsuperscript{12} through Arg\textsuperscript{18} are located closer to the micelle surface, though they still are near the micelle interior. These results suggest that each side of the hairpin prefers different micelles. To simplify the discussion, the strand that includes residues 1 through 8 will be referred to as strand 1 and the strand from residues 11 to 18 will be referred to as strand 2. Strand 2 is preferentially imbedded in DPC, while strand 1 is located closer to this micelle’s surface. In SDS, however, this is reversed: strand 1 prefers the micelle interior while strand 2 prefers to be more near the surface.

In both systems, the arginine residues are in locations around the lipid head groups, at the micelle–water interface. These residues likely drive the peptide to interact with the membrane and prefer to remain in the head group region as it allows them to interact with negatively charged groups.

Radial Distribution Functions and Hydration Numbers

Radial distribution functions (RDF) were calculated between the peptide and the micelle. The data were averaged over the last 500 ps of simulation for the DPC system and the last 300 ps of the SDS system. Radial distribution functions illustrate relative affinities between different sets of atoms in each system.

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* Values in parentheses are RSMD.
RDFs between the side chains and the negatively charged group on the micelle heads and also between the side chains and the micelle core atoms were calculated. The two systems show similar RDFs for each residue with the head groups (Figure 8). There are strong interactions between the arginine residues and the SDS sulfate and DPC phosphate groups. The arginine residues show somewhat stronger interactions with DPC than with SDS (Figure 8A and B). This difference can be attributed to the location of the negatively charged lipid head group with respect to the micelle surface. In SDS, the negatively charged group is much closer to the surface, so the arginines also interact with water. In DPC, the phosphate group is buried by the choline groups, shielding arginines from water.

Two residues that show distinctly different interactions with the head groups are Val14 and Val16 (Figure 8C and D). Val16 is more attracted to the DPC head groups, and Val14 is more attracted to the SDS head groups.

The radial distribution functions calculated for the side chain atoms with the hydrophobic chains of the micelle interiors also show a preference for different sides of the hairpin with the two interiors. The plot of the RDFs for Leu5 shows a dramatic difference between its interactions with SDS versus DPC (Figure 9A). There is no peak for Leu5 with the DPC interior atoms, though it has a significant interaction with SDS. Phe12 and Val14 show the opposite trend; that is, strong interaction with the DPC interior but no interaction with the SDS hydrocarbon core (Figure 9C and D).

Hydration numbers were also calculated, as a method of quantifying the interactions between the peptide and water. Hydration numbers are similar to radial distribution functions in that they tell how many water molecules are located near the residue, thereby giving information as to whether a residue is located in the bulk (high hydration numbers) or in the micelle (low hydration numbers). Two sets of hydration numbers were calculated for each system, one for the side chain atoms, and another for the carbonyl carbon of each residue as shown in Table II. Both sets exhibit the same trend of more hydration in SDS than in DPC. In SDS, the least hydrated side chains are the cysteine residues and Leu5. This, along with the position of Leu5 in the interior of the micelle suggests that it might play a key role in the antimicrobial activity of PG-1. More of the carbonyl carbon atoms are less hydrated in the SDS

**FIGURE 6** Interaction energies calculated for the hydrophobic residues of PG-1 and the two micelles. It is clear that there is a difference in the attraction between the residue Leu5 and the two types of micelles and also for Phe12. Leu5 shows preference for SDS, as evidenced by its more negative value, likewise for Phe12 with DPC.
system than the side chains, specifically the residues from Arg through Arg, excluding Tyr. In DPC, the side chains that are most significantly nonhydrated are the cysteine residues and more importantly Phe, Val, Val, and Gly. These same residues show low hydration numbers for the carbonyl carbon.

FIGURE 7 Electron density profiles in SDS and DPC (running averages). The profiles show the locations of specific groups in the system. Most interesting to note is the order of the residues in the hydrophobic face are opposite for DPC and SDS, suggesting different modes of interaction for PG-1 with the two types of micelles.
The purpose of this molecular dynamics simulation study is to provide a high-resolution atomistic picture of the interactions of PG-1 with different types of micelles and to explain the peptide’s activity and toxicity profiles in terms of differential behavior in its interactions with mammalian and bacterial membrane mimics. The simulation results can be compared to several laboratory studies of PG-1 in lipid bilayers.

One of the most interesting differences between the interaction of PG-1 in DPC and SDS is the location of the peptide with respect to the micelle surface. Though the peptides appear to move to the same position based on center of mass distance data (see Figure 4), in actuality, PG-1 remains largely inside the DPC micelle as this micelle has a larger radius, while PG-1 moves to the surface of the SDS micelle. The final configuration of the peptide in the DPC system is in agreement with the proposed location of PG-1 in a DLPC bilayer. The orientation was measured using solid-state NMR spectroscopy experiments. The experiments used peptides labeled on either the $^{13}$CO or the amide $^{15}$N of Val$_{16}$. Val$_{16}$ is labeled because it is neither in the turn region nor at the termini. Shown in Figure 10 is the orientation that Yamaguchi et al. predicted from these experiments. PG-1 tilts to allow the arginine residues at the two ends of the molecule to interact with the head groups. We see a similar interaction in the DPC micelle, where the region surrounding Val$_{16}$ and also the corresponding region on the opposite strand interact strongly with the micelle hydrocarbon core. It does seem that our simulations agree with the experiments in that the final conformation allows Phe$_{12}$ and Val$_{14}$ to be far from the PC head groups, while still allowing for the interactions between the head groups and the arginine residues.
The peptide moves much faster in SDS than in DPC in the four simulations performed here. This is probably due to the high negative charge on the surface of SDS. The peptide is more intensely attracted to its final position in SDS due to its cationic arginine residues. In DPC, the positive charges of the zwitterionic group screen the electrostatic interactions. It is not clear that this behavior can be extrapolated to the kinetics of the peptide interaction with bacterial and mammalian cell walls. Although the electrostatic interactions will be similar, the very slow relaxation times of lipids compared to relaxation times of the micelle molecules might dominate the time scales of peptide–membrane interaction kinetics.

The lack of depth of insertion of PG-1 into the SDS micelle may be due simply to the size and curvature of the micelle. The peptide and micelle are still interacting strongly, as evidenced by the high level of disruption of the micelle shape (30% to 45% distortion from the pure micelle in terms of moments of inertia), and there are sections that are clearly imbedded into the micelle interior. PG-1 in SDS is significantly more hydrated than in DPC; however, there are areas that are not hydrated. In particular, the residues on strand 1 show much weaker interaction with the water subphase and stronger interactions with the micelle core.

Qu and coworkers noted a well-ordered, nonpolar cluster involving Leu, Tyr, Phe, Val, and Val that extend above the plane of the β-sheet. This region is in the structurally constrained and amphipathic central β-sheet portion of PG-1, which is considered to be the critical antimicrobial component. This face provides a point of reference for some interesting interactions with the micelles in the simulations. Comparing electron density profile derived locations, it becomes evident that the face interacts in drastically different ways with the two types of micelles. In SDS, the order of residues from inside out is Leu, Tyr, Phe, Val, and Val. In DPC, the order is Phe, Val, Val, Tyr, and Leu. This

FIGURE 9 Radial distribution functions for selected residues with the hydrophobic micelle core.
In SDS, there is a stronger interaction between Leu and the hydrophobic core, as there is with Cys; however, there are significantly stronger interactions for Phe and Val with the core of DPC.
means that strand 2 prefers the interior of DPC, while strand 1 prefers the surface. The reverse is true in SDS. This is also reinforced by radial distribution function data, which show a strong preference for Leu\(^5\) with the SDS micelle interior, but no preference for this region from Phe\(^{12}\), Val\(^{14}\), or Val\(^{16}\), and the opposite preferences for PG-1 in DPC.

It has been proposed that protegrins work by disrupting cell membranes,\(^6\) and the effect that PG-1 has on the SDS micelle in this simulation supports this. The disruption of the integrity of the micelle illustrates the significance of the interaction between the peptide and micelle. The change of up to 45\% in the ratio of moment of inertia for SDS with the peptide compared to a pure SDS micelle is especially dramatic and suggests that the presence of Leu\(^5\) in the interior of the micelle along with interactions of the rest of the peptide at the surface can account for PG-1’s antimicrobial activity.

A possible mechanism of interaction for PG-1 with bacterial membranes can then be proposed as follows: the initial strong electrostatic attraction of the peptide to the micelle surface is followed by insertion of strand 1 into the hydrophobic core. Specifically, the arginines in PG-1 are attracted to the negatively charged head groups. This is supported by the interaction energy data that show the strong electrostatic interactions. This could pull the peptide and hold it in place at the surface, allowing the hydrophobic residues of the peptide to be in close contact with the micelle interior. Eventually this results in the disruption of the micelle.

That the two systems exhibit such drastically different results provides information about the ability of protegrins to differentiate between bacterial and mammalian membranes. Strand 2 (RF\(^{12}\)FC\(^{14}\)VC\(^{16}\)GR) is significantly more hydrophobic than strand 1 (RG\(^{12}\)GL\(^{14}\)CR\(^{16}\)) and appears to have a preference for the core of the DPC micelle over the core of the SDS micelle. Importantly, this differential behavior suggests possibilities for improving the toxicity and activity for use in humans. Changing Phe\(^{12}\) or Val\(^{14}\) may possibly cause a significant change in hemolysis and toxicity of PG-1 without affecting the antimicrobial activity of PG-1. It appears that strand 1 is more important in activity against bacterial membranes; therefore, if this strand is kept constant, the peptide should retain its antibacterial activity. From the simulations, it appears that the ideal (active against microbes and harmless to host cells) peptide should have the amino acid sequence of strand 1 intact and a modified strand 2 made less hydrophobic.

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