Original Article

Investigation on the Effects of Bactenecin on POPC Membrane in Atomistic Details Using Molecular Dynamics Simulation

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Abstract

Background: Traditional antimicrobial agents are losing their efficiency as microbial resistance increases. Thus, developing antimicrobial peptides (AMPs) can assist as an alternative approach. For AMPs, the hypothesis mode of action is involved in pore formation within the lipid membrane, thereby leading to cell death. In this study, interaction between Bactenecin and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine (POPC) was studied. **Methods:** For this purpose, two systems, Bactenecin in water and Bactenecin in POPC were treated by 50 ns of molecular dynamic simulation and data were compared with those of free POPC. **Results:** The results suggest that the interaction between Bactenecin and bilayer membrane cause some disorder and more instability along with little compactness of bilayer. The hydrogen bond between peptide and heads of lipid components may is main reason of membrane compactness. The results can provide some information on how to Bactenecin or other such peptides affect bio-membranes.

Keywords: *Antimicrobial peptides, bactenecin, bilayer membrane, molecular dynamic simulation, POPC*

Introduction

Antimicrobial peptides (AMPs) are one of the most important categories of antimicrobial agents which continued to be the subject of attention during the past years because of the rising resistance of pathogen bacteria against common antibiotics.[1]

In the recent years, scientists worldwide have made significant efforts to introduce antibacterial peptides such as hybrid peptides of CM11 (WKLFKKILKVL-NH2) and CM15 (KWKLFKKIGAVLKVL-NH2),[2] buforin II,[3] and magainin[4] which act mainly on the bilayer membranes. These information has obtained using the analysis changes of membrane in the presence of peptide.[5,6] Nowadays, a lot of effective AMPs were introduced around the world, hundreds of which exist in living organisms such as fungus, plants, and animals.[7,8] In the recent years, however, the efforts for making peptides with 30–40 residues length have been performed in large number of laboratories.[9]

When antibiotics were first used successfully in treating infectious diseases, many scientists believed that humans have finally succeeded curing infectious diseases forever. However, after some decades and because of drug resistance, this group of medicine will no longer control over infectious diseases.[10] Antibiotics are mostly used among patients while its overdose can be dangerous due to drug resistance and/or common side effects.[11]

AMPs related to cathelicidin are a family of polypeptides in macrophages, polymorph nuclear leukocytes, and keratinocytes.[12] Cathelicidins have an important role in the defense system of mammals against bacterial infections.[13] The peptides related to this family are known as AMPs. They were first found in neutrophil but later were seen in other cells such as epithelial and macrophage, after activation by bacteria, viruses, and fungus, or by 25-hydroxyl-vitamin-D13-alpha-hydroxylase hormone.[12] Bactenecin highly tends to interact with the bacterial membrane, especially those of *Escherichia coli*, and also to penetrate into its lipopolysaccharide. This interaction with the cytoplasm

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membrane causes the membrane to elapse and finally, the cellule dies. This ability has been identified by dipropylthiacarbocyanine.[14,15]

Molecular dynamics (MDs) simulations have an important role in providing atomic descriptions of many biological phenomena, a description that is often hard, if not impossible, to obtain experimentally.[16,17] This technique, which provides an atomic resolution model of the system of interest, has also been employed extensively to study AMP – biomembrane interactions.[16,18,19] Especially, simulations have been applied successfully to explore pore formation induced by AMPs.[20,21]

Because of the importance of investigation on novel antimicrobial agents, in this study, the bactenecin peptide will be inserted in the bilayer lipid membrane of palmitoyl-oleoylphosphatidylcholine (POPC) as a model, and its probable effect on the membrane will be investigated using the powerful method of MD simulation.

Methods

Molecular dynamics simulations

Basic structure of a sheep bactenecin was extracted from 4JWE pdb code of RSCB protein database. All the steps of the simulation were performed by GROMACS 5.1.0. A GROMOS 53a6 force field was used for all calculations.[22] The basic structure of POPC bilayer and the parametric files for GROMACS were taken from Tieleman (http://moose.bio.ucalgary.ca). Periodic boundary conditions were applied in all directions. A cutoff of 1 nm was applied for electrostatic interaction measurements using particle mesh ewald (PME) method.[23] Short range cutoff of van der Waals energy was adjusted in 1.0 nm in the MD simulation. The NVT and NPT ensembles were used to fix temperature and pressure in 300 K and 1 bar using Berendsen *et al*. thermostat[24] and Parrinello and Rahman barostat,[25] respectively. All bonds were treated by linear constraint solver method to constrain around their equilibrium distance. Finally, a 50 ns of MD simulations was performed using leapfrog algorithm. Molecular structures and graphics were generated by VMD is a freeware from Illinois University. (www.ks.uiuc.edu/Research/vmd, University of Illinois at Urbana-Champaig, USA), as well.[26]

Bactenecin/water system

Simulation of peptides in water was done by putting the bactenecin molecules in a cube sized of 6.441 nm × 5.903 nm × 6.575 nm followed by solvation with 5150 water molecules of Extended Simple Point Change (SPC/E) model of water. This system was neutralized by adding four atoms of chlorine.

Bactenecin/POPC system

The bactenecin were put in a membrane which consists of 128 molecules of POPC and four chlorine atoms, and 3355 water molecules, and will be called bactenecin/POPC from now. The InflateGro method is used to make a cylindrical-shaped hole in the biobilayer membrane around the bactenecin. The axis is placed vertically on the biobilayer.

Free POPC system

A free POPC system also was simulated in order to compare its changes with those of POPC in the presence of bactenecin.

Results and Discussion

To investigate probable effects of antibacterial peptide of bactenecin on lipid biomembranes, MD simulations were applied. Investigating root mean square displacement (RMSD) [Figure 1] has implied that all systems reached stability after the equilibrium time. As can be seen in Figure 1, the curves reach to a stable plateau after 20 ns. In bactenecin/water system, it has increased up to about 3 nmin <10 ns and after that has stabilized. In bactenecin/POPC system, the RMSD values have increased up to 3.5 nm and then have remained constant. The obtained results confirmed the well-equilibrated systems for performing further analysis. As in literature by Klepeis *et al*.,[27] a higher RMSD corresponds to a more unstable structure and a lower RMSD represents a more stable peptide. These differences propose that the bactenecin has different overall dynamic behavior throughout the MD simulation in two studied systems maybe because of more flexibility of peptide in the lipophilic medium of the membrane.

Radius of gyration (Rg) is a measure of the amount of compactness or opening in the given structure.[28] Rg values in both Bactenecin (BAC)/POPC and BAC/water have not changed significantly and reached to a balance of 8.5 A° after about 35ns of simulations [Figure 2]. These results show that this is no difference in peptide length neither in water nor in lipids. Considering this result along with the more values of RMSD for peptide in bilayer medium, it can be concluded that the amino acid side chains undergo some displacement in membrane medium.

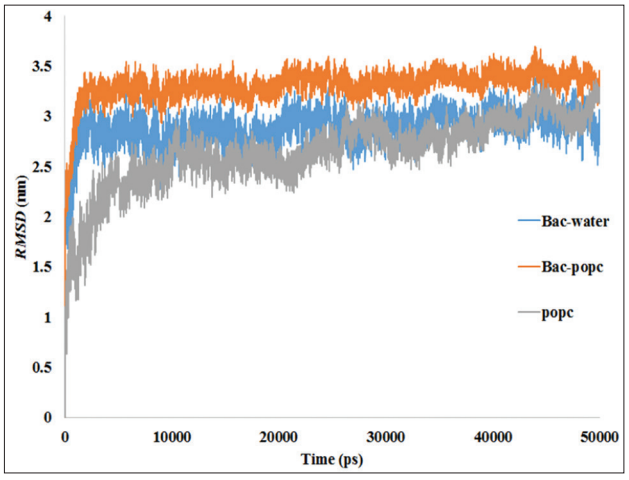

Figure 1: Evolution of Cα root mean square deviation during the molecular dynamics simulation for studied systems

Root mean square fluctuation (RMSF) has been studied in both systems during MD simulations. As it can be seen in Figure 3, the RMSF values of bactenecin in POPC are higher than bactenecin in water. The rising trend in RMSF of bactenecin in POPC is a result of more fluctuation and mobility of peptide in lipids. Analyzing RMSF analysis shows that fluctuations are more close to C and N terminals of bactenecin. About the values related to peptide in bilayer medium, those hydrophilic leucine and proline residues which exist in the middle of peptide have even bigger fluctuations than others. These vast fluctuations of peptide can also cause instability in biomembrane and destroy its native configuration.

As well as the covalent bonds and salt bridges, hydrogen bonds (H-bonds) are among the most key interactions between macromolecule and its environment.[29] H-bonds have different levels of strength from nearly as strong as covalent bonds to as weak as van der Waals interactions.[30] In a particular peptide, the H-bonds which act as connectors provide plenty of strong units.[29,31] The bactenecin is studied regarding the H-bonds in the POPC bilayer biomembrane [Figure 4]. The conditions $r \leq 0.35$ nm and ≤60° were used as criteria for a H-bond to exist (a H-bond was assumed to exist if the donor-acceptor groups' distance was not more than 0.35 nm and the angle of donor-hydrogen-accept or triplet was not more than 60°). There are two arginine residues which can have more important roles relative to other residues in forming the H-bond with the biobilayer membrane. Figure 4 shows that the H-bond between bactenecin and POPC has increased up to 20 during 50 ns simulation. These H-bonding interactions can cause consolidation of peptide within the membrane and may induce the persistent effect of peptide on bilayer.

Deuterium order parameter is a phase transition factor during simulation. It also allows conclusions in fission distribution, even in very complicated conditions.[32] The graph in Figure 5 shows that the bactenecin peptide is placed in the membrane which makes the membrane to have more organization compared to the free POPC. This can lead to sol to gel switching and more rigidity of biomembrane.

The density of the membrane was investigated in the bactenecin containing versus free POPC system and the results are depicted in the graphs in Figures 6a and b, respectively. Based on considered co-ordinate, the results could be different, and so, the favored co-ordinate for this purpose is the z-axis. From these results, it can be concluded that the density of the different parts of system has no more changes when they are compared. On the other hand, there are some shifts in local density related to head groups in peptide-containing system that move to center of box. These changes can be because of compressing bilayer by peptide, leading to lowering of bilayer thickness.

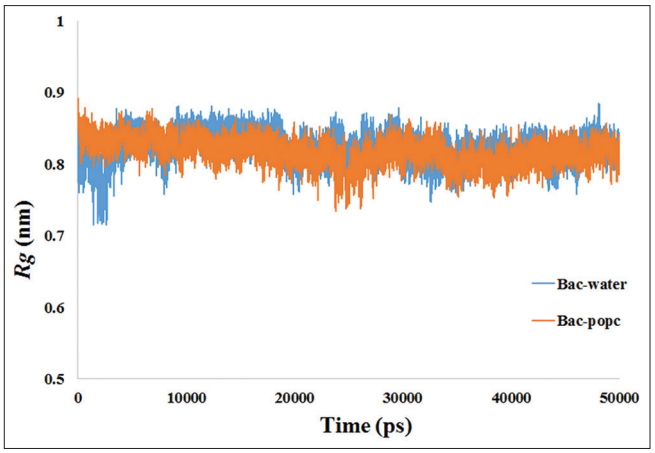

Figure 2: Time dependency of evaluated radius of gyration of peptide in water and biobilayer

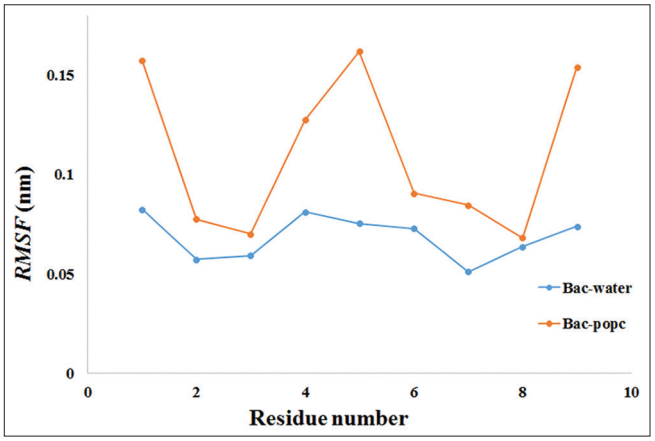

Figure 3: The root mean square fluctuations of peptide residues in respect to their time-averaged values

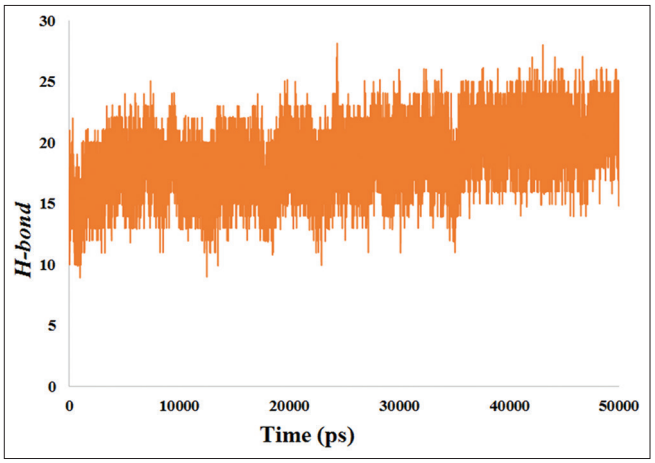

Figure 4: Changes in the total number of hydrogen bonds of peptide–lipid

There are natural movements in the biobilayer membrane because of its liquidity, and anything that can destroy this membrane property can cause instability and loss of its natural function. The analysis of mean square displacement (MSD) can measure the lateral diffusion or flip-flop movants of biobilayer lipids and may be

used to ensuring the membrane to save its natural mobility.[33,34] Figure 7 shows the decreasing of MSD in the bactenecin-POPC system than free POPC during the simulation. This result is an indication of lesser diffusion of lipids in the presence of peptide and abnormality in the POPC membrane caused by bactenecin. These results are in good agreements with those of deuterium order that suggests freezing effects of peptide on biomembrane.

The area per lipid is an essential parameter for describing the state of molecular packing within a lipid bilayer. In this study, the average area per lipid molecule was estimated by multiplying the XY dimensions of the simulation box and divided by the number of lipid molecules present in one leaflet of the bilayer. Results show that at the end of simulation, the value is 65.8 A^2 for free POPC system and 62.3 A^2 for the membrane containing peptide. This finding shows some compactness in peptide-containing system during simulation time that can be because of bactenecin effects.

Bilayer thickness analysis checks the thickness of the biobilayer membrane during simulation. The thickness is high around the peptide before the simulation, and it decreases after it. Figure 8 shows that in the last of simulation, the thickness of the peptide-embedded membrane is less than those of related to the beginning. The reason for this falling may be stretching the phospholipid head groups by bactenecin. These results also are in good agreement with H-bonding and density analysis. As mentioned above, there were some H-bonding interaction of bilayer with peptide that can be because of stretching and decreasing in membrane thickness.

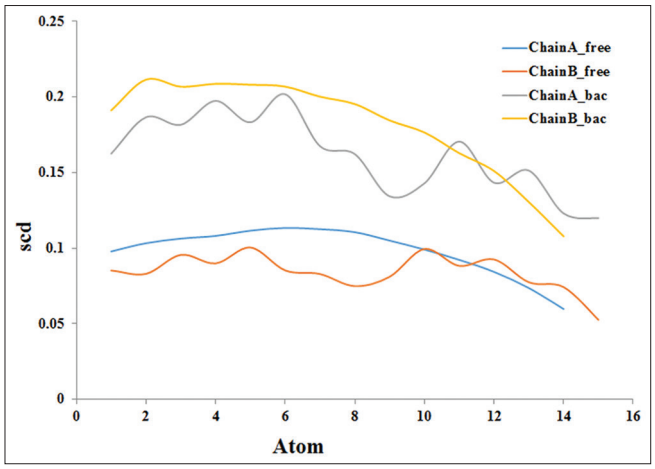

Figure 5: Order parameters of the POPC lipid bilayers in free and peptide-embedded conditions

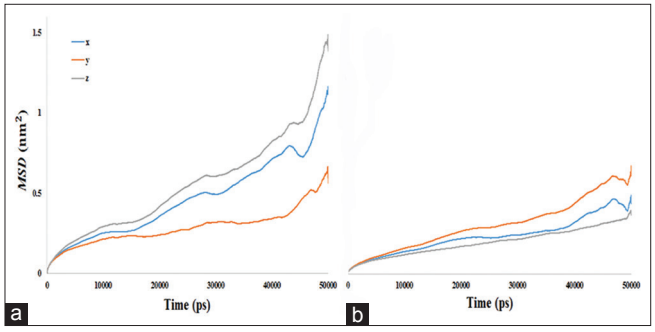

Figure 7: The marginal penetration of lipids in x, y, and z axes for free POPC (a) and peptide-embedded POPC (b)

Conclusion

In this study, important interactions and effects of AMPs of bactenecin on the POPC bilayer have been investigated using all-atom MDs simulation. The results show that RMSD value for peptide in POPC medium is more than those of in water and that can be because of more displacements of bactenecin in membrane. Peptide has made H-bonds with the heads of lipids and this makes the membrane more compact and the leaflets went close together after simulation. The presence of bactenecin in membrane causes instability of lipids and the liquidity index was decreased. Furthermore, insertion of peptide can cause more ordering in bilayer lipids and makes it more

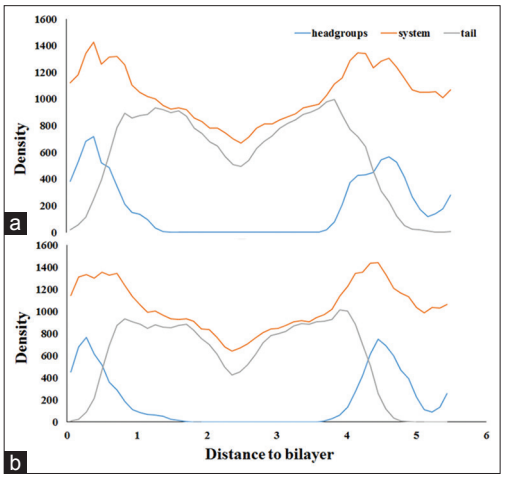

Figure 6: Changes in the density of different components of free POPC (a), peptide POPC (b) systems

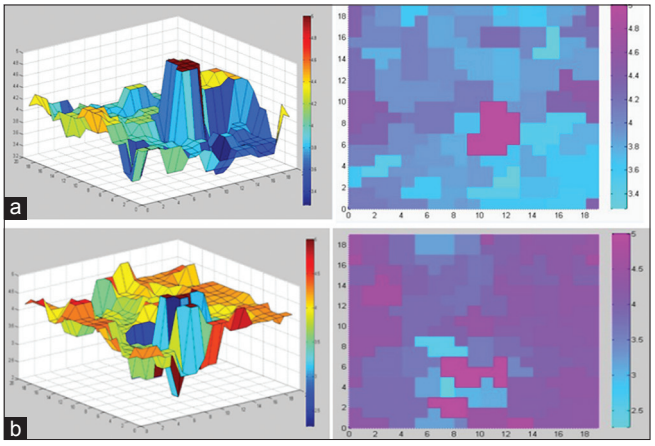

Figure 8: Thickness bilayer of free (a) and peptide-embedded (b) systems of biomembrane POPC

rigid. The bactenecin used in this study can be a part of more complex models in further studies. Overall, in this study, the harmful effects of bactenecin on the lipid bilayer membrane (POPC) are proved and it can be a confirmatory of its potential usage as antimicrobial agent.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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