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ORIGINAL ARTICLE

Effect of Linear and Cyclic *Lysine-Lysine-Tryptophan- Tryptophan -Lysine-Phenylalanine* Antimicrobial Peptide on Sodium Dodecyl Sulfate Micelle as Cell Membrane Mimetic: Molecular Dynamics Simulation Study

S. Hassan Mortazavi, Mohammad Reza Bozorgmehr*, Mohammad Momen-Heravi

Department of Chemistry, Mashhad Branch, Islamic Azad University, Mashhad, Iran

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KEYWORDS

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ABSTRACT: Drug resistance has limited the synthesis of new antibiotics. Therefore, the use of compounds that do not have drug resistance has been considered. Antimicrobial peptides are among the compounds for which drug resistance has not been reported. On the other hand, it has been found that the activity of these compounds is less than that of antibiotics. Therefore, the design of appropriate antimicrobial peptides is challenging. To address this challenge, efforts have been made to understand their mechanism of action. However, their mechanism of action is not well understood. In this work, the interaction of two cyclic and linear antimicrobial peptides with sodium dodecyl sulfate micelles as cell membrane mimetics method has been studied by molecular dynamics simulation. The micellar radius of gyration shows good agreement with the experimental results and the results of other simulations performed. Calculation of the conformational factor shows that cyclic antimicrobial peptide has a greater affinity for interaction with micelles. Charged and aromatic residues are involved in the interaction of cyclic antimicrobial peptides with micelles. Whereas, only charged residues are effective in the interaction of the linear antimicrobial peptides with micelle.

INTRODUCTION

Today, the widespread use of drugs has made bacteria resistant to them [1]. On the other hand, the synthesis of antibiotics, which are effective compounds against bacteria, has been limited [2]. Therefore, the study of antimicrobial peptides has been considered [3]. Antimicrobial peptides are structurally low molecular weight (about 10 kDa) with a limited number of residues. The nature of antimicrobial peptides is usually cationic. Although the activity of these compounds against bacteria is not high, bacterial resistance to them has not been reported so far [4].

Degradation of cell membranes and degradation of eliminating intracellular cytoplasmic targets are two mechanisms proposed for the function of antimicrobial peptides [5]. In the second mechanism, to destroy cytoplasmic organs, the antimicrobial peptide must pass through the membrane and enter the cell. Thus, the

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*Corresponding author: bozorgmehr@mshdiau.ac.ir, mr_bozorgmehr@yahoo.com (M. R. Bozorgmehr)

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common aspect of the two mechanisms is the interaction of the antimicrobial peptide with the membrane [6]. Many studies have been performed on the interaction of antimicrobial peptides with membranes. The results of this study show that antimicrobial peptides destroy cell membranes in two ways: by creating holes in the membrane and by destabilizing the membrane [7]. Because there are lysine and arginine residues in the structure of antimicrobial peptides that have a positive charge, these residues interact the negative charges of the membrane. This provides an opportunity to orient the side chain of the aromatic residues of the antimicrobial peptide towards the membrane. Phenylalanine and tryptophan are among the aromatic residues of antimicrobial peptides [8]. The side chains of these residues are hydrophobic in nature, which can interact with the lipid membrane and destabilize it.

Despite much information available on the mechanism of action of antimicrobial peptides, there is still much ambiguity. For example, which mechanism occurs, membrane destruction or destruction of cytoplasmic organs or a mixture of both? The antimicrobial effect of cationic peptides on membranes that are not charged has also been observed. Therefore, the hypothesis that positive charges first interact with negative membrane charges is questionable [9, 10]. Also, it has been shown that antimicrobial peptides have a different mechanism of action than their linear form if they are cyclic [11].

In this work, the linear and cyclic structure of antimicrobial *Lysine – Lysine – Tryptophan - Tryptophan – Lysine - Phenylalanine* (AMP: anti-microbial-peptide. C-AMP as cyclic and L-AMP as linear) peptide has been studied by molecular dynamics simulation and density functional theory. Sodium dodecyl sulfate micelle was used to mimic the effect of the antimicrobial peptides on the membrane.

MATERIALS AND METHODS

Since there is no direct experimental information to compare the interaction of linear and cyclic AMP with sodium dodecyl sulfate, the simulations were divided into two parts. In the first part, a micelle of 60 molecules of sodium dodecyl sulfate was designed. The designed micelle was placed in the center of a simulation box. Water molecules were randomly placed around the micelle to fill the box. Since sodium dodecyl sulfate has a negative charge, sodium ions were added to neutralize the designed system. The ions were randomly added to the simulation box. The edges of the designed simulation box are 1 nm away from the surface of the micelle. The reason for selecting 60 molecules for sodium dodecyl sulfate micelles is that this number is close to the coordination number of sodium dodecyl sulfate at its critical micelle concentration [12]. This number has also been used in other simulations to allow comparisons [13]. Single point charge (SPC) Type water molecules were used to fill the simulation box. Web server Micelle Maker was used to build micelles [14]. Figure 1 shows the designed simulation box with its contents.

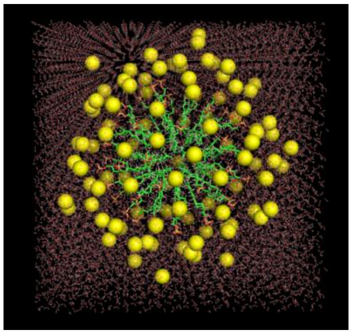

Figure 1. Structure of sodium dodecyl sulfate micelles in a cubic simulation box. Sodium ions are shown in yellow and water molecules in red. Sodium dodecyl sulfates are green and in the middle of the box.

The protocols presented in references [15] and [16] were used to perform a molecular dynamics simulations of the designed system at this stage. Because the force field parameters of sodium dodecyl sulfate molecule are not provided by default in the molecular dynamics simulation software, these parameters were calculated using the PRODRG web server [17]. To create an input on the PRODRG web server, the optimized structure of the sodium dodecyl sulfate molecule is needed. The structure of this molecule was optimized by the B3LYP density functional theory method and 6-31G basis set. To ensure optimization of structures, frequency calculations were performed using the same method and the same previous function. The results showed that there are no virtual frequencies for the optimal structures. The calculations of this step were done using the GAMESS software [18]. The results of this step of the calculations were used to control the simulations performed.

In the second part of the calculations, 2 simulation boxes were created like the simulation box designed in the previous step. with the difference is that in the boxes designed at this stage, C-AMP and L-AMP were randomly placed. The initial structure of C-AMP was taken from the protein database with the code 1skk [19]. L-AMP molecule was designed manually and its structure was optimized using the above method used for the optimization of the sodium dodecyl sulfate molecule (Figure 2).

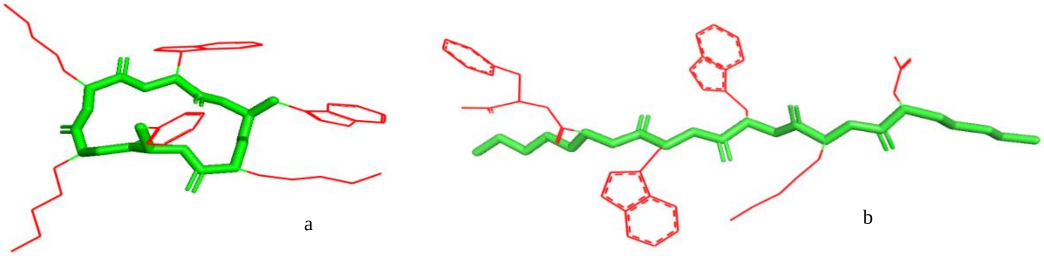

Figure 2. Structure of (a) C-AMP and (b) L-AMP. Backbone highlighted as sticks and green and sidechains highlighted as line and red.

Gromacs software version 5.1.2 Gromos 43a1 force field was used for the calculations [20]. To eliminate inappropriate contacts between atoms, the steepest descent algorithm was used to optimize the designed systems. Then, in two steps with simulation time 1 ns and time step 2 fs, each of the designed systems achieved equilibrium in NVT and NPT ensembles, respectively. In the final step, molecular dynamics simulation was performed for 100 ns at the same time step as the equilibration step. The V-rescale and Berendsen algorithms were used to control the temperature and pressure of the system components, respectively [21]. For these weak-coupling algorithms, a coupling time of 1.0 ps was considered. The chemical bonding of non-solvent components was fixed with the LINCS algorithm [22] and the chemical bonding of solvent molecules with the SETTLE algorithm [23]. To test the convergence of the obtained results all simulations were repeated 3 times. Particle Mesh Ewald (PME) was used for calculating the total electrostatic energy. Lennard-Jones model with a cutoff distance of 10 Å was used to calculate other non-bonded interactions.

RESULTS AND DISCUSSION

Two quantities, the radius of gyration (Rg) and micelle moment of inertia are quantities that have been experimentally reported for sodium dodecyl sulfate micelles. The Rg is the radius of the smallest sphere that surrounds the micelle. Therefore, it is a measure of the shape of the micelle. The radius of gyration (R_g) of the micelle was obtained using the following equation 1:

$$R_g = \left(\frac{\sum_i \|r_i\|^2 m_i}{\sum_i m_i} \right)^{\frac{1}{2}} \qquad (1)$$

Where the atomic mass of i (m_i), and atomic position of i (r_i) are dependent on the center of mass of a molecule. The value of Rg and the ratio of moment of inertia for micelles were calculated from the last nanosecond of the simulation and the results are listed in Table 1.

Table 1. The value of Rg and the $\frac{I_{max}}{I_{min}}$ of micelle containing 60 molecules of sodium dodecyl sulfate.

	Rg	$\boldsymbol{\frac{I_{max}}{I_{min}}}$	**Ref.**
This work	1.565	1.180	This work
MD-a	1.567	1.210	[15]
MD-b	1.630	1.040	[24]
MD-c	1.600	1.020	[13]
Experimental	1.450	-	[25]

According to this table, a good agreement is found between the obtained results and other simulation data, and the experimental value.

According to the results obtained from the simulations of the second part, sodium dodecyl sulfate molecules form micelles that interact with the peptide were identified. To do this, 60 molecules of sodium dodecyl sulfate were numbered in the micelle structure. The conformational factor, P_i, which is a measure of the tendency of interaction between two molecules, was then calculated. The conformational factor was calculated based on the method developed by Housaindokht et al [26]. The conformational factor is the mean contact with a specific molecule over the simulation time, which can be obtained from the $= \frac{n_I}{<N>}$. And <N> is obtained from the following equation 2:

$$< N >= \sum_{i=1}^{N} \frac{n_i}{N} \qquad (2)$$

Where n_i is the number of contacts with the molecule i and N is the molecule number in the micelle structure. The P_i conformation factor represents high values of affinity relative to the other molecule when it's greater than 1.

The sodium dodecyl sulfate molecule with P_i >1 can be considered as the binding site, and when P_i <1, the affinity to the peptide is not observed. The conformational factor values for sodium dodecyl sulfate molecules were calculated and the results are listed in Table 2.

As can be seen from the Table, the number of sodium dodecyl sulfate molecules with a conformational factor greater than 1 is greater for C-AMP than for L-AMP. This result indicates that the cyclic antimicrobial peptide interacts more with the membrane than the linear peptide. This observation is consistent with the results obtained in other sources [11]. To better explain the observed results, the simulations performed in step 2 were sampled. (Figure 3)

Table 2. The conformational factor values of sodium dodecyl sulfate (SDS) in presence of C-AMP and L-AMP.

SDS-*index*	$P_{i,C-AMP}$	$P_{i,L-AMP}$	SDS-*index*	$P_{i,C-AMP}$	$P_{i,L-AMP}$
1	0.025	0.008	31	1.489	0.880
2	0.029	0.088	32	1.556	0.089
3	0.035	0.099	33	1.660	0.092
4	0.044	0.128	34	1.675	0.109
5	0.056	0.209	35	1.776	0.239
6	0.050	0.331	36	0.998	0.365
7	0.066	0.452	37	0.896	0.455
8	0.099	0.033	38	0.769	0.369
9	0.258	0.049	39	0.601	0.235
10	0.665	0.123	40	0.789	0.326
11	0.901	0.368	41	0.896	0.568
12	0.896	0.377	42	0.699	0.685
13	0.999	0.488	43	0.528	0.698
14	0.867	1.258	44	0.482	0.771
15	0.664	1.389	45	0.581	0.851
16	0.699	1.456	46	0.662	0.991
17	0.991	1.690	47	0.528	0.867
18	1.098	1.789	48	0.458	0.823
19	1.129	1.896	49	0.523	0.663
20	1.390	1.991	50	0.428	0.666
21	1.456	0.965	51	0.651	0.566
22	1.526	0.689	52	0.421	0.667
23	1.669	0.771	53	0.369	0.781
24	1.891	0.862	54	0.259	0.658
25	2.021	0.654	55	0.532	0.458
26	2.123	0.369	56	0.654	0.586
27	1.998	0.568	57	0.784	0.268
28	1.991	0.698	58	0.881	0.348
29	2.256	0.668	59	0.756	0.651
30	1.888	0.865	60	0.658	0.884

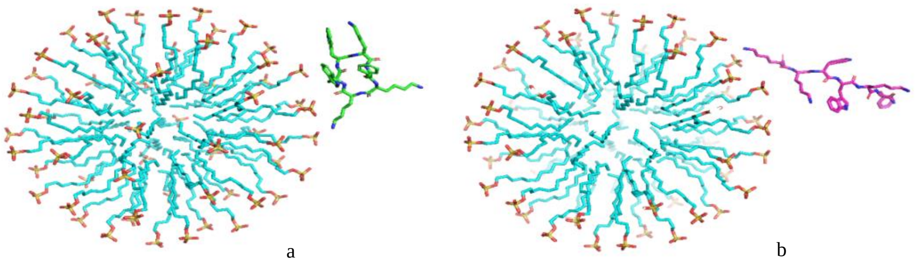

Figure 3. Micelle structure along with (a) C-AMP and (b) L-AMP.

As can be seen from the Figure, the cyclic antimicrobial peptide interacts with micelles through its charged and aromatic residues. While linear antimicrobial peptide only interacts with micelles through its positively charged residues.

CONCLUSIONS

Antimicrobial peptides are compounds that replace antibiotics to reduce drug resistance. These compounds perform their therapeutic action by destroying the cell membrane or destroying the cytoplasmic organ. A common aspect of the mechanism of action of microbial peptides is their interaction with membranes. However, this mechanism is not yet fully understood. Molecular dynamics simulation is a method that allows the study of process mechanisms at the atomic and molecular scales. In this research, the molecular dynamics simulation method has been used to study the interaction of an antimicrobial peptide with a membrane. Sodium dodecyl sulfate micelles were considered as mimic cell membranes. The antimicrobial peptide was designed in two cyclic and linear forms. The results of this study showed that the cyclic form of antimicrobial peptide is more active. Charged and aromatic residues are involved in the interaction of the cyclic antimicrobial peptide with the membrane. In cases where there was experimental data, the results of this study show a good agreement with this data.

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Conflict of interests

All authors of this research paper have declared that there is no conflict of interest.

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