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Membrane insertion profiles of peptides probed by molecular dynamics simulations

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Abstract

Membrane insertion of small peptides plays important roles in antimicrobial defense, toxin actions, and viral infections. There have been experimental efforts to study this process with carefully designed synthetic peptides. Molecular dynamics simulation techniques are useful tools to study membrane insertion of peptides in atomic details to complement these experimental efforts. We developed a methodology based on molecular dynamics simulation techniques to probe the insertion profiles of small peptides across the membrane interface. The peptide is represented in full atomic detail, while the membrane and the solvent are described implicitly by a generalized Born model. To sample peptide conformations across the membrane interface, we apply an umbrella sampling technique, where the center of mass position of the peptide is restrained at various positions across the membrane interface. Free energy profiles are calculated as a function of the peptide position with respect to the membrane center and structural deviations from the native structure by the weighted histogram analysis method. We applied the methodology to a synthetic peptide mimicking the transmembrane domain of the M2 protein from influenza A virus. Two different initial peptide conformations, one fully extended and the other helical, have been used to probe the effect of peptide structures on the membrane insertion mechanism. A larger free energy decrease was observed when the peptide inserts into the membrane in a helical conformation than when it enters membrane in a non-helical conformation. We discuss an improvement of the current methodology by increasing the sampling of peptide conformations with replica-exchange molecular dynamics simulations. With a growing number of bacterial infections that are resistant to conventional antibiotics, small peptides based on naturally-occurring antimicrobial peptides have become attractive candidates for a new class of antibiotics. The current methodology is expected to be useful in the design and engineering of therapeutic agents based on antimicrobial peptides with specific membrane-insertion profiles.

1. Introduction

Membrane insertion of small peptides plays important roles in antimicrobial defense, toxin actions, and viral infections [1-3]. With the proper understanding of the membrane-insertion process of peptides or proteins, countermeasures against bacterial and viral diseases could be developed by designing synthetic peptides with specific membrane-insertion profiles [4, 5]. However, membrane insertion profiles

of peptides are dependent on their amino-acid sequence composition and complex interactions with lipid membrane and aqueous solution. Extensive studies with a diverse set of model peptides are needed for a better understanding of the membrane-insertion process. Structure and dynamics of membrane-bound peptides have been partly investigated experimentally with carefully designed small synthetic peptides (7, 8). However, despite significant advances in experimental techniques, only a limited number of membrane protein structures have been experimentally determined [6], and characterizing large conformational changes of small, membrane-bound peptides during the membrane-insertion process remains a significant challenge. Molecular dynamics (MD) simulation techniques are useful computational tools to study membrane insertion of these small peptides in atomic details to complement these experimental efforts [7]. Computer simulations of membrane insertion of peptides have been performed based on various models of membranes and proteins ranging from full all-atom to coarse-grained models [8-13].

Here, we present a methodology based on MD simulation techniques to probe membrane-insertion profiles of small peptides across the membrane interface. A better understanding of the sequence-structure relationship of peptides in the membrane environment gained by the application of the current methodology will enable us to design peptides with specific membrane-insertion profiles. In the current methodology, the membrane-insertion profile of a peptide is estimated by calculating the position-dependent potential of mean force (PMF) [14] from MD simulations. For an efficient sampling, biased sampling techniques with peptide-position restraints are used as in a recent MD simulation study of the thermodynamic stability of a charged arginine in a transmembrane helix [15]. We applied the current methodology to the M2 transmembrane peptide (M2-TMP), a synthetic peptide mimicking the transmembrane domain of the M2 protein from influenza A virus with a sequence SSDPLVVAASIIGILHLILWILDRL. The M2 protein from influenza A virus forms a four-helix tetrameric bundle that exhibits proton ion-channel activity required for the proper release of genetic material from the endosome [16]. The M2-TMP monomer in the membrane environment has been studied

experimentally [17] and computationally [11-13, 18]. However, a complete picture of the structural and energetic changes of the M2-TMP upon penetrating the membrane from an aqueous solution is still missing. We find that the membrane-insertion of a peptide in a helical conformation is more favorable than in a non-helical conformation in accordance with the traditional view of peptide insertion.

2. Methods

MD simulations were performed with the CHARMM program version c31b2 [19]. We used the all-atom PARAM22 force field with CMAP modification to represent the M2-TMP [20, 21]. The membrane and the water solvent were represented by an implicit membrane/solvent model implemented in the generalized Born with a switching window (GBSW) module [18]. The GBSW implicit membrane model has been successfully applied to study membrane-insertions of various model peptides [8]. The membrane thickness of 25 Å and the surface tension coefficient of 0.04 kcal/(mol Å2) have been used to represent the dimyristoylphosphatidylcholine (DMPC) membrane [8, 18], which was used in the experimental study [17]. Membrane smoothing lengths of 0.6 and 5 Å were used for simulations with and without peptide-position restraints, respectively.

Two different starting configurations of the M2-TMP, a fully extended conformation and an ideal α-helix structure as determined by solid-state NMR techniques [22], were prepared after brief energy minimizations. A time step of 2 fs has been used in the MD simulations. Langevin dynamics with a friction coefficient of 5.0 ps^{-1} has been used for the temperature control [8]. Covalent bonds between the heavy atoms and hydrogens were constrained by the SHAKE algorithm [23]. Distances used for the onset of a switching function for non-bonded interaction, the cutoff for non-bonded interactions, and the cutoff for non-bonded list generation were 20, 22, and 25 Å, respectively. Coordinates were saved at every 1 ps for further analysis.

In simulations with the peptide-position biasing, the *Z*-component of the center-of-mass position of a peptide with respect to the membrane center was restrained by the harmonic potential with a force

constant of 2 kcal/(mol $Å^2$) using the GEO command in the Miscellaneous Mean Field Potential (MMFP) environment in CHARMM. The *Z*-axis is defined as the membrane normal with the membrane center as its origin. The weighted histogram analysis method (WHAM) [24] described by Gallicchio et al. [25] has been used to remove biases due to peptide positions and obtain free energy profiles of membrane insertion of the peptide. We calculated the PMF as a function of the peptide *Z* position of the peptide and the root-mean square distance (RMSD) with respect to the α helix structure. RMSD between two structures is defined as the square-root of the minimum average square distance between respective backbone atoms of the two structures with respect to all rigid body rotations and translations [26].

As will be described in more detail along with our results, we performed 208 MD simulations, 162 with and 46 without the peptide-position restraints, with the total simulation time of 1.5 microseconds, using roughly 24000 CPU hours on Linux clusters at the U.S. Army Research Laboratory Major Shared Resource Center.

3. Results

3.1. MD simulations of the M2-TMP inside and outside of the membrane

We performed 32 MD simulations of the M2-TMP pre-inserted perpendicularly into the membrane with a fully extended conformation as shown in Figure 1 (a). The simulations were maintained at the same temperature of 300 K but started with different initial atomic velocities. Each simulation lasted 20 ns except for 3 simulations that were discontinued at 10, 16, and 18 ns. Figure 2 (a) and (b) show the average RMSD from the experimental helical conformation and the *Z*-position (perpendicular height above the center of the membrane) of the peptide as a function of time, respectively. In all 32 simulations, the center of mass of the M2-TMP remained near the membrane center. However, the

conformations of the peptide remained substantially non-helical for the duration of the simulation as shown by the distribution of RMSD in Figure 2 (c).

We also conducted MD simulations of the M2-TMP starting from a fully extended conformation located outside the membrane at 45 Å away from the membrane center lying parallel to the membrane interface as shown Figure 1 (b). Fourteen different MD simulations were performed at temperatures starting at 300 K and at each 50 K temperature increment up to a high of 950 K. Each simulation lasted 5 ns except for the temperature of 700 K which was discontinued at 4 ns. In all MD simulations, no spontaneous insertion of the M2-TMP has been observed, and the M2-TMP remained outside the membrane at an average distance of 56 Å from the membrane-center. The RMSD distribution shown in Figure 2 (c) indicates that structures of the M2-TMP remain deviated from the experimentally determined helical conformation. However, Figure 2 (c) also reveals that there is considerable overlap between the structural conformations generated at trajectories performed at different temperatures. Hence, the lower RMSD structures (more helical) can be accessible from MD simulations at higher temperatures. These results show that better sampling techniques, such as replica-exchange molecular dynamics (REMD) [27] simulations, may help sampling such structural transformations as demonstrated in recent studies [11, 18].

3.2. Free-energy MD simulations with peptide-position biasing

To sample peptide conformations at a wider range of points across the membrane/water interface, we performed MD simulations of the M2-TMP with its center-of-mass *Z*-position restrained at multiple locations across the membrane interface. Two different initial peptide conformations, one fully extended and the other helical, have been used to probe the effect of peptide structures on the membrane insertion mechanism. The peptide was initially oriented parallel to the membrane interface in both types of starting conformations. The center-of-mass *Z*-position of the peptide was restrained at 81 points across the membrane interface in intervals of 0.5 Å starting at the center of the membrane ($Z = 0$ Å) and ending at Z

= 40 Å. Only the upper half of *Z*-positions ($Z \geq 0$ Å) was considered because the peptide-membrane interaction is expected to be symmetric with respect to the membrane center ($Z = 0$ Å). The simulations lasted 5 ns and the last 2 ns of simulation trajectories were used for the analysis. We removed the biasing imposed by the *Z*-position restraints with the WHAM analysis and calculated PMF surfaces as a function of *Z*-positions and RMSD.

The PMF surface in Figure 3 (a), obtained from simulations that were started from the helical conformation, appears to indicate that the M2-TMP inserts itself into membrane as a helix while undergoing minor structural deviations from the helical conformation. One dimensional PMF profiles with respect to the *Z*-position (obtained by averaging over all RMSDs) in Figure 3 (a) shows that the free-energy decrease slows down after crossing into the interfacial region from the aqueous phase. A small free energy barrier exists at near $Z = 6.8$ Å. However, there is a clear free energy minimum at the membrane center. The PMF surface in Figure 3 (b) calculated from simulations that were started from an extended conformation appears to suggest that the M2-TMP readily moves near to the interface as indicated by a significant free energy decrease (~30 kcal/mol) going from the aqueous phase ($Z = 40$ Å) to the interface ($Z = 12.5$ Å). However, both 2D and 1D PMF profiles show a very little free energy decrease from the interface to the membrane core in a non-helical conformation compared to the insertion in a helical conformation.

As estimated from the difference in PMF values at $Z = 40$ Å and 0 Å in the 1-D PMF profile in Figure 3 (a), the free energy of the M2-TMP is decreased by 56 kcal/mol when it is inserted into a membrane as a helical conformation. However, the corresponding free energy decrease estimated from the PMF profile in Figure 3 (b) for simulations started with an extended conformation is 29 kcal/mol. These results indicate that the free-energy change for a hypothetical insertion of the M2-TMP in a helical conformation is more favorable than the insertion in non-helical conformations. Similarly, the internal energy changes of the peptide going from $Z = 40$ Å to $Z = 0$ Å are -56.7 and -30.8 kcal/mol when simulations were started from a helix and from an extended conformation, respectively. At the membrane

center (Z = 0 Å), the internal energy of the M2-TMP as calculated from simulations whose initial starting conformation was a helix is 51.7 kcal/mol lower than that from simulations starting from an extended conformation.

The PMF surfaces in Figure 3 show that these MD simulations sampled only conformations close to their starting structures and failed to show a minimum for the partially inserted M2-TMP. This lack of convergence between results with different initial conformations of the M2-TMP suggests insufficient sampling associated with the constrained 5-ns MD simulations. A similar lack of conformational sampling from straight-forward applications of MD simulation was also observed in the results shown in Figure 2. In the current set of simulations, although a harmonic potential was applied to restrain the Z-position of the peptide, it is possible that 5-ns MD simulations may not be enough to sample all the possible structural conformations at the given peptide position. However, our results suggest that these free-energy MD simulations can provide useful information on the relative free-energy change of the M2-TMP during the membrane insertion for a given set of different peptide conformations despite the shortcomings in the sampling of peptide conformations.

4. Discussion

In this study, we introduced a methodology to calculate the membrane-insertion profile of an atomically-detailed peptide. The aqueous solution and the bilayer-membrane itself were treated in the continuum approximation using a generalized Born model. We applied this methodology to calculate a membrane-insertion free energy profile of the M2-TMP. To enhance samplings of peptide conformational states, we performed MD simulations with peptide positional restraints. A larger amount of free energy decrease was observed when the peptide inserts into the membrane in a helical conformation than when it enters membrane in a non-helical conformation in agreement with the broadly accepted model on the mechanism of the mechanism of peptide membrane-insertion [28]. However, MD simulations at constant

temperatures with and without peptide positional restraints displayed a lack of proper sampling of conformational states of the M2-TMP by showing strong dependencies on the initial starting conformations. These simulations failed to find the transformation of a fully extended peptide into the experimentally known helical conformation even when it was pre-inserted into the membrane. These findings underscore the importance and difficulties of sampling enough conformational states to obtain a meaningful and statistically significant membrane-insertion free-energy profile from MD simulations. The current methodology may be improved by increased sampling of peptide conformations with REMD simulations.

With a growing number of bacterial infections that are resistant to conventional antibiotics, small peptides based on naturally-occurring antimicrobial peptides have become attractive candidates for a new class of antibiotics. The current methodology is expected to be useful in the design and engineering of therapeutic agents based on antimicrobial peptides with specific membrane-insertion profiles.

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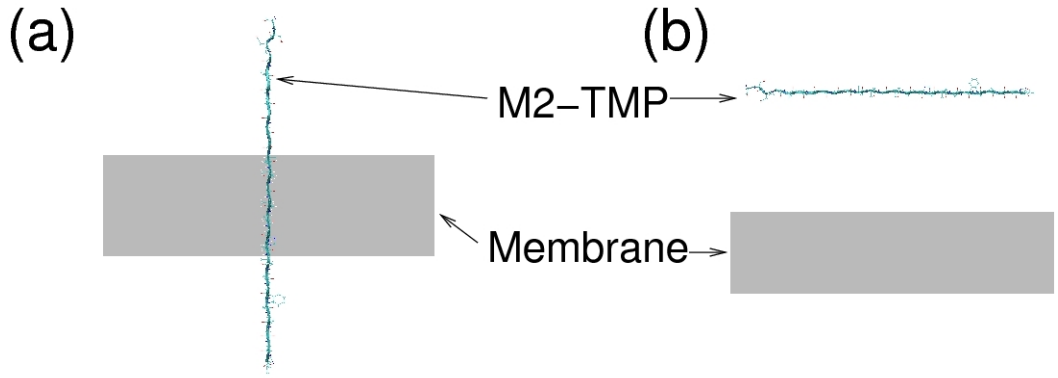

Figure 1:

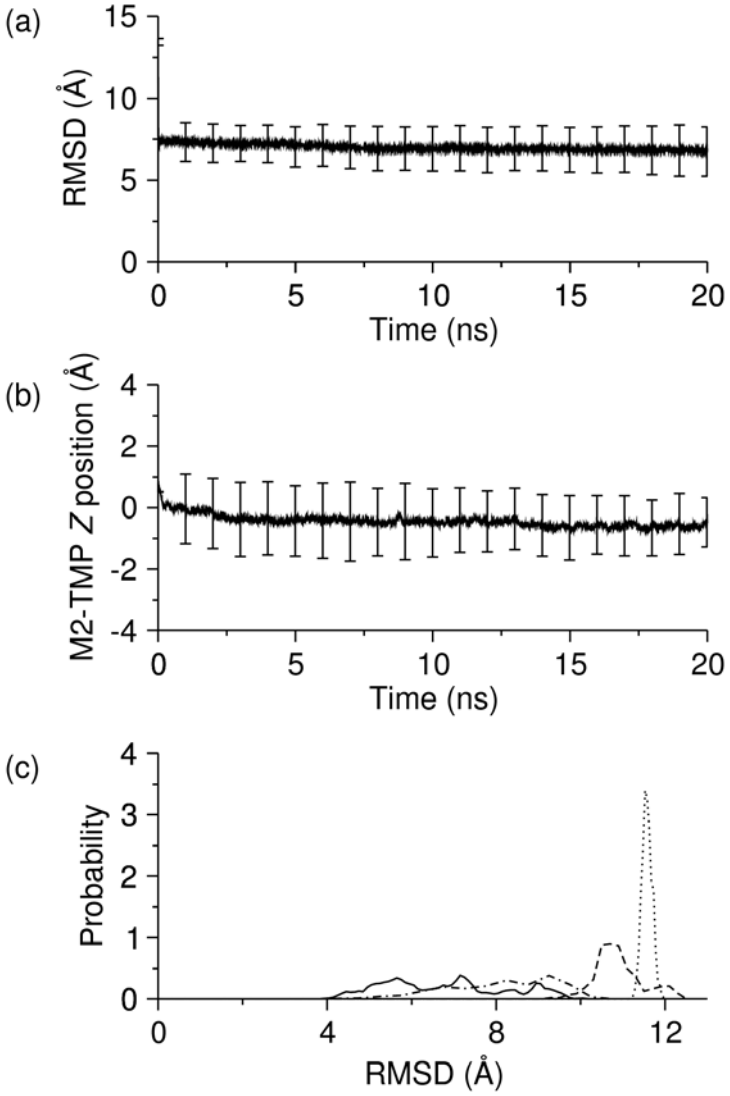
(a)
RMSD (Å)
Time (ns)
(b)
M2-TMP Z position (Å)
Time (ns)
(c)
Probability
RMSD (Å)

Figure 2:

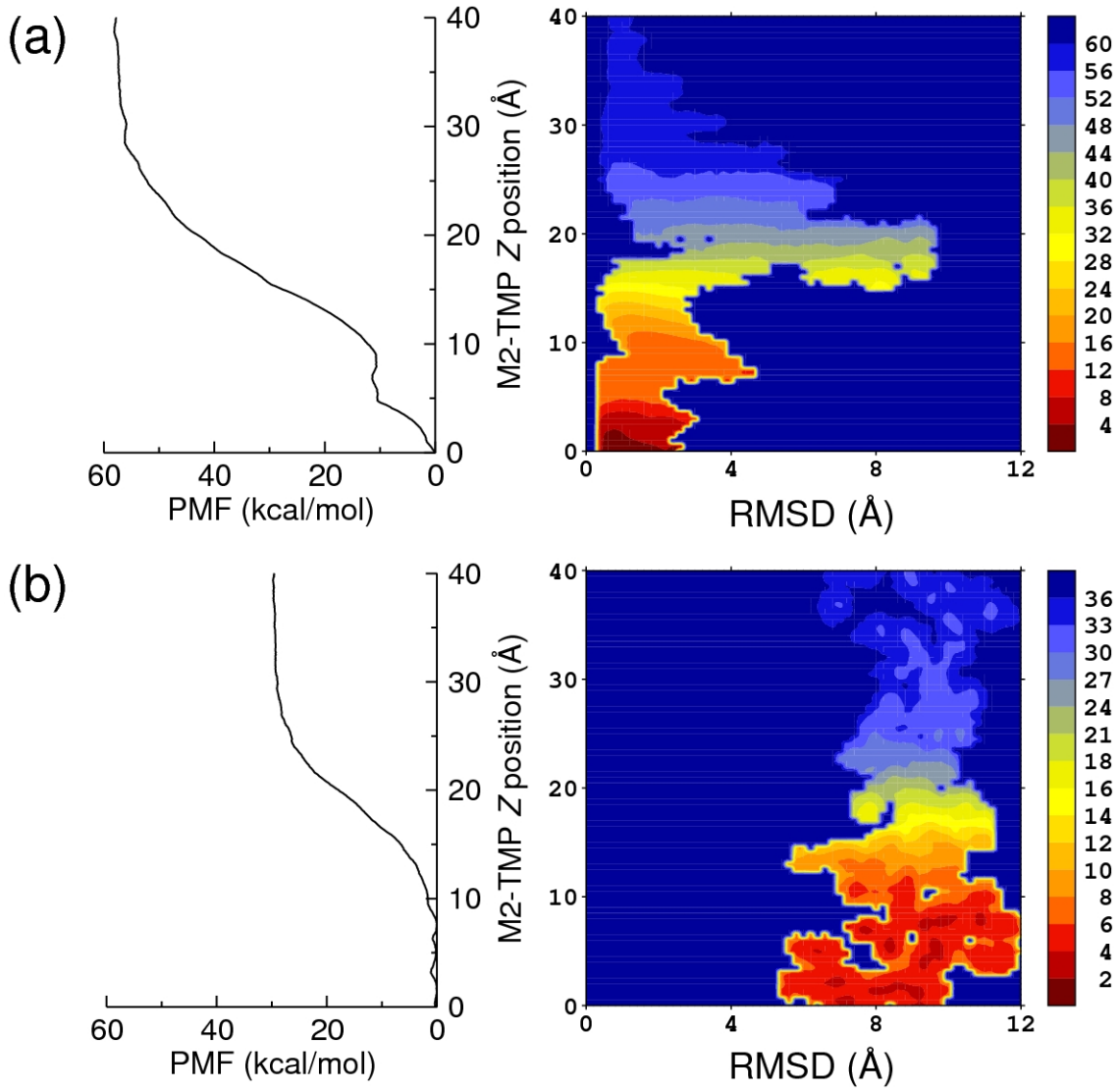
(a)
PMF (kcal/mol)
M2-TMP Z position (Å)
RMSD (Å)
(b)
PMF (kcal/mol)
M2-TMP Z position (Å)
RMSD (Å)

Figure 3:

Figure Legends

Figure 1

Two different, with respect to the membrane, starting configurations of the fully extended M2-TMP; (a) The M2-TMP is pre-inserted perpendicularly into the membrane, (b) The M2-TMP is initially placed outside, but parallel to the membrane surface.

Figure 2

(a) The average RMSD of the M2-TMP pre-inserted into the membrane with an extended conformation as shown in Figure 1 (a), as a function of the simulation time calculated from 32 separate room-temperature MD simulations each started from a set of initially different velocities. Variations in the data are indicated by the standard deviations ($\pm\sigma$) calculated at each time step. After an initial drop the ensemble average RMSD remains steady at about 6.8 Å. (b) Same as in (a) but for the center-of-mass *Z*-position of the M2-TMP. The average position remains near the center of the membrane throughout the simulations. (c) Distribution of RMSD values with respect to the initial helical conformation calculated from the last 1 ns of trajectories from all MD simulations. A solid line gives the distribution of RMSD values for trajectories derived from the initially extended conformation pre-inserted perpendicularly into the membrane. Distributions of RMSD value from trajectories of peptides initially placed outside the membrane, as shown in Figure 1 (b), at temperatures of 300, 500, and 800 K are represented by the dotted, dashed, and dot-dashed lines, respectively.

Figure 3

One-dimensional PMF profiles as a function of the center-of-mass *Z*-position and 2D PMF surfaces as a function of RMSD (with respect to the helical conformation) and the *Z*-position obtained from free-energy MD simulations of the M2-TMP. The peptide center-of-mass *Z*-position is restrained at various locations extending from the center of the membrane to well outside the membrane. For each restrained simulations the distribution of conformations are calculated and the final PMF surface is pieced together by the WHAM analysis. These simulations were started from two sets of initial configurations and the resulting PMF surface are shown for simulations initiated with (a) a helical conformation and (b) an extended conformation. The PMF surfaces are thus very dependent on the initial conditions.