The effects of solvent and temperature on the structural integrity of monomeric melittin by molecular dynamics simulations

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Abstract

In this study, the secondary structural integrity of monomeric melittin was shown to depend strongly on the solvent by 200 ps molecular dynamics simulations with temperature jump technique. The \( \alpha \)-helix content of melittin increased with increasing the aliphatic chain length of alcohol and decreased with increasing simulation temperature. In addition, the melting temperature of melittin, at which the averaged helicity decreased to 50%, was linearly correlated to the aliphatic chain length of alcohol. The weaker dielectric constant of longer aliphatic chain length of alcohol possibly reduces the hydrogen bonding between amide protons and surrounding solvent molecules and simultaneously promotes the intramolecular hydrogen bonding in melittin and therefore stabilizes the secondary structure of melittin.

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1. Introduction

Melittin, the major protein component of the venom of the European boney bee *Apis mellifera*, exhibits a powerful hemolytic activity. This amphipathic peptide has a net charge of +5; four of which are in the highly basic C-terminal tetrapeptide sequence K21–R22–K23–R24, with only K7 in the N-terminal region. Melittin adopts different conformations and aggregation state depending on several factors including peptide concentration, pH, ionic strength, and the nature of the negative counterion [1]. No detectable secondary structure is observed by circular dichroism (CD) or \(^1\)H NMR when melittin is in aqueous monomeric form [2,3]. The tetrameric form of melittin, on the other hand, consists of monomers predominantly in a helical conformation [4,5]. The conformational and aggregational properties of melittin in water seem to result from two opposing forces; promoting self-association is the hydrophobic effect that acts to sequester nonpolar amino acid residues in the interior of proteins and opposing self-association are the high positive-charge density of melittin and the
entropic term associated with the formation of stable secondary structure in the self-associated tetramer [6].

The structure of the aqueous melittin tetramer has been solved by X-ray crystallography [7,8]. In addition, the averaged solution structures of monomeric melittin in methanol, and in dodecylphosphocholine micelles have also been determined using high-resolution proton NMR and amide exchange analysis [9,10]. The degree of helicity of crystalline tetrameric melittin is greater than expected from the values determined by CD [3,5] probably due to the high ionic strength and peptide concentration used in crystallization. In addition, melittin is monomeric and α-helical in methanol [9,10]. The structural information shows that backbone amide hydrogens in the N-terminal sequence from residues 5 to 11 and in the C-terminal sequence from residues 16 to 23 are stable to exchange with solvent, indicating protection by hydrogen bonding in a stable helical structure. In contrast, the amide in a central turn of helix from residues 12 to 15 are weakly protected from exchange indicating that the N- and C-terminal helical segments are connected by a flexible turn of helix with unstable helical hydrogen bonds. In addition, the NMR and amide-exchange studies emphasize the effect of the central P14 in enhancing flexibility at the center of the helical structure which seems to act as a ‘hinge’ allowing the helix to bend to accommodate conformational constraints induced by intermolecular interactions [11].

Temperature jump technique has been considered to be the simplest approach to accelerate the protein unfolding rate in silico. Usually, temperatures in the range of 400–600 K are usually employed. Although the unfolding events may be much more complicated, it still significantly reduces the time required for unfolding simulations to the ps or ns timescale [12]. Previously, we have successfully applied MD simulations to investigate the behavior of various complex biomacromolecules [13,14]. In the present study, 200 ps MD simulations were conducted to investigate the solvent and temperature effects on the structural integrity of monomeric melittin with temperature jump technique.

2. Materials and methods

The X-ray crystallographic structure of melittin was taken from Protein Data Bank (PDB entry: 2MLT) as a dimeric structure [7,8]. In order to avoid the association effect of melittin and to protect the helical structure, the molecular mechanics calculations were carried out using monomeric structure of melittin (Fig. 1). The molecular minimization and dynamics simulations were carried out using Discover 2.9.8 program in the SGI O200 workstation with 64-bit HIPS RISC R12000 2 × 270 MHz CPU and PMC-Sierra RM7000A 350 MHz processor (Silicon Graphics, Mountain View, CA, USA) in the consistent valence forcefield (CVFF) [15]. The dynamics trajectories were analyzed using ANALYSIS module of Insight II program (Accelrys, San Diego, CA, USA). The minimum image periodic boundary conditions (PBC) were used to keep the constant volume during the MD courses. The range of cut-off radius was set as 14 Å for both non-bonded electrostatic and van der Waals interactions.

The monomeric structure of melittin was submitted to 2000 energy minimization iterations using conjugate gradient method and then used as the initial structure for subsequent MD simulations. The solvent boxes of different alcohols were determined based on room temperature density and molecular weight by calculating the volume of the unit cell for each alcohol molecule. Each unit cell of alcohol was equilibrated by performing 500 steepest descent minimization and 3 ps dynamics calculations with an augment box of 6×, 7×, and 6× for A, B, and C axis of the unit cell, respectively. The explicit image PBC was used for solvent equilibrium since any molecule migrates out of the solvent box will not be re-imaged at the end of the equilibrium when minimum image was used. At the end of explicit image equilibrium, Discover will re-image molecule whose center of mass has moved out of the solvent box in order to maintain the integrity of the solvent box with a relatively constant density.

The initial structure of monomeric melittin was placed in the center of a pseudo-unit cell with the size of 60 × 35 × 35 Å³ and soaked with various alcohols into the unit cell. The dimensions of these
cells were similar to the previous study [16]. In all of the solvated melittin simulations, the counter ions (Cl\(^-\)) were added around the five positive residues to obtain a neutral simulation system. In order to arrange the soaked alcohols randomly, solvent molecules alone were submitted to 10,000 iterations by conjugate gradient minimization, keeping the peptide atoms fixed to avoid the change of melittin structure during the solvent randomization step. The system composed by the minimized solvent molecules and the monomeric melittin was used as the starting image since the solvent molecules and melittin have both achieved their well energy-minimized structures. Then, 200 ps MD simulations with 5 ps in equilibrium step were carried out under various solvent conditions with constant temperature constraint at 300, 400, and 600 K. The integration step in all simulations was 1 fs and the trajectories of melittin structures were saved every 2 ps for further analysis. The room-mean-square deviation (RMSD) of each saved structure was determined after optimal superimposition of the coordinates to the energy-minimized crystallographic structure to remove translational and rotational motion. In addition, the secondary structure analyses were based on the theory proposed by Kabsch and Sander [17]. The helicity was defined as the ratio of the number of the residual H bonds at time \( t \) to the number of the total H bonds in the two \( \alpha \)-helices of the initial structure of melittin. The averaged helicity was then defined by \( \frac{1}{t_f} \int_0^{t_f} H(t) \, dt \), with \( t_f \) as the total length of the MD simulation. The melting temperature, \( T_m \), was defined as the temperature at which the averaged helicity decreased to 50%.

3. Results and discussion

In this study, the effects of temperature and solvent on the structural integrity of melittin were evaluated by plotting the \( C_z \) RMSDs of melittin as a function of running time. Fig. 2 shows that \( C_z \) RMSDs increased more significantly when melittin was placed in pure water or pure methanol system as temperature increased from 300 to 600 K. It indicates that water played a negative role on maintaining melittin in its native helical conformation. In addition, the structural integrity of melittin increased as the number of carbon atoms of alcohols increased, implying that a solvent with less polarity seemed to stabilize the helical structure of melittin to a greater extent. In solvent of low polarity, hydrophobic interactions stabilizing the native structure are weakened, and simulta-
neously the local hydrogen bonds are strengthened, resulting in stabilization of the extended \(\alpha\)-helical structures. However, the exact mechanism of the alcohol-induced \(\alpha\)-helix formation of proteins is still unclear. Previous study has shown that the curve of hydrogen bond strength versus increasing TFE concentration matches both in shape and magnitude the increase in average helix propensity in TFE/water mixtures [18]. Consequently, strengthening the hydrogen bonds is possibly responsible for the alcohol effects on the helix formation of short peptides. The results of this study showed that alcohols with lower dielectric constants or more CH groups should closely approximate the interior of proteins and also should strengthen interactions between charged groups, thus decreasing the hydrogen bonding of amide protons to the solvent and hence strengthening intramolecular hydrogen bonding. The net result of this effect is the stabilization of \(\alpha\)-helix. Our results are consistent with the previous findings that the efficiency of enhancement of helicities increases with the aliphatic chain length of the alcohol [19,20].

The secondary structure propensity of melittin was predicted according to the Kabsch and Sander algorithm [17] during the entire MD courses. As shown in Fig. 3, the \(\alpha\)-helix content increased with increasing the aliphatic chain length of the alcohol and decreased with increasing temperature. As observed from the RMSD results (Fig. 2), secondary structure analysis (Fig. 3) also showed that water and methanol are likely to induce the destruction of the native helical structure of melittin. Fig. 3 also showed that the N-terminal \(\alpha\)-helix of melittin (residues 2–10) is more unstable than the C-terminal one (residues 12–26). The charged polar side chains of some amino acid residues usually have positive effect on stabilizing \(\alpha\)-helix due to the enhancement of intramolecular hydrogen bonds along the polypeptide chain [21]. The helicity is positively correlated with the number of charged residues in \(\alpha\)-helix [21], thus introducing charged amino acids into the primary structure of proteins and providing the environment with low dielectric constant are the most effective methods in increase the structural integrity of \(\alpha\)-helix. Previous study also showed that the charge–charge interaction of Arg and Lys residues is strongest when they are located in \(i\) and \(i + 4\) positions on the same face of the helix [22]. There are four charged residues (i.e., K21–R22–K23–R24) located on the C-terminal \(\alpha\)-helix of melittin, whereas only one charged residue (i.e., K7) is found on the N-terminal \(\alpha\)-helix. Thus, the higher secondary structure stability of the C-terminal \(\alpha\)-helix against the N-terminal \(\alpha\)-helix is probably due to the charge–charge interactions between these charged residues. In addition to the stabilizing effect by the charge–charge interactions, the interaction between \(i\) and \(i + 4\) aromatic side chains has also been shown to stabilize \(\alpha\)-helix conformation by hydrophobic interactions, electrostatic interactions, and van der Waals forces [23]. Shi et al. [24] have indicated that the polar–nonpolar interaction of Trp and Arg located in \(i\) and \(i + 4\) positions strongly stabilize \(\alpha\)-helix. The similar polar–nonpolar interaction
may occur between W19 and R22 in the C-terminal α-helix of melittin, thus increasing its structural integrity. Although W19 and R22 are located in i and i + 3 positions instead of i and i + 4 mentioned by Shi et al. [24], they are located on the same face of melittin and still form possible polar-nonpolar interaction as shown in Fig. 1.

To determine the stability of the secondary structure of melittin in various solvents, the averaged helicity was calculated during the 200 ps MD simulations and the results are given in Table 1.

### Table 1
Averaged helicity and melting temperature of melittin in various solvent and temperature

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Averaged helicity (%)</th>
<th>$T_m$ (K)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>300 K</td>
<td>400 K</td>
</tr>
<tr>
<td>Water</td>
<td>0.606</td>
<td>0.198</td>
</tr>
<tr>
<td>MeOH</td>
<td>0.552</td>
<td>0.308</td>
</tr>
<tr>
<td>EtOH</td>
<td>0.709</td>
<td>0.240</td>
</tr>
<tr>
<td>PrOH</td>
<td>0.642</td>
<td>0.460</td>
</tr>
<tr>
<td>BuOH</td>
<td>0.829</td>
<td>0.569</td>
</tr>
</tbody>
</table>

The averaged helicity decreased dramatically as temperature increased from 300 to 600 K in pure water. In all temperature examined, melittin remained the highest averaged helicity in pure butanol. Table 1 also shows the melting temperature, $T_m$, of the secondary structure of melittin in various solvents. Apparently, $T_m$ increased as the aliphatic chain length of the alcohol increased. The linear relationship between $T_m$ and the aliphatic chain length of the surrounding alcohol is shown in Fig. 4.

![Fig. 3. Secondary structures as a function of MD simulation time for melittin at 300, 400, and 600 K in: (a) water; (b) methanol; (c) ethanol; (d) propanol; and (e) butanol. The numbers shown on the y-axis in each plot indicate the number of amino acid residues. α-Helix, turn, and coil estimated according to the Kabsch and Sander algorithm [17] are shown in red, blue, and green, respectively.](image)

![Fig. 4. Linear relationship between the melting temperature of melittin and the aliphatic chain length of the surrounding alcohol.](image)
chain length of the alcohol was thus plotted in Fig. 4. It indicates that the stabilizing effects of alcohols on \( \alpha \)-helix increases with the aliphatic chain length of the alcohols.

Previous MD simulations have shown that the stabilizing effect of TFE is induced by the preferential aggregation of TFE molecules around the peptides [25]. This coating displaces water, thereby removing alternative hydrogen-bonding partners and providing a low dielectric environment that favors the formation of intrapeptide hydrogen bonds [25]. Indeed, clustering of alcohol molecules is an important factor that enhances the effects of alcohols on proteins and peptides. All the simulations in the present study were carried out in pure alcohol systems to eliminate the effect of water, thus we may assume that melittin was covered by alcohol clusters as in the TFE/water system. Our results showed that the structural integrity of melittin increased with de-

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![Fig. 5. Snapshots of melittin in: (a) water; (b) methanol; (c) ethanol; (d) propanol; and (e) butanol during the entire 200 ps MD simulations.](image-url)
creasing dielectric constant of the solvent, indicating that there is a positive correlation between the number of carbon atoms in alcohols and the stability of melittin [26]. The results of this study were consistent with the previous studies, in which methanol was shown to have little contribution to the alcohol-induced α-helix formation of melittin by measuring the ellipticity at 222 nm [27].

The snapshots of melittin in various solvents at 600 K during the 200 ps MD simulations were shown in Fig. 5. It is obviously that melittin lost its structural integrity very quickly both in water and in methanol, whereas propanol and butanol seemed to have the highest stabilizing effects on maintaining melittin in its helical structure. The results indicate that the structural integrity of melittin depends strongly on the solvent. The structure of monomeric melittin is mostly unstable in a solvent with high dielectric constant, thus it is likely to aggregate to form homotetramer structure in water by hydrophobic interaction.

In conclusion, the α-helix propensity and melting temperature of monomeric melittin were shown to increase with increasing the aliphatic chain length of the alcohol and decreased with increasing simulation temperature. The effect of various alcohols on stabilizing melittin is most likely due to their low dielectric constant, favoring the formation of intramolecular hydrogen bonds instead of intermolecular hydrogen bonds and promoting the formation of secondary structure.

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**References**