The Stability of Alpha-Helix of the Helical Antimicrobial Peptide in Polar/Apolar Solvent

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Abstract: G(XXKK)nX-CONH2 series antimicrobial peptides have broad-spectral bactericidal activity, high killing rate. The antimicrobial sensitive are affected by many factors. In this article, three cationic amphiphilic peptides G(XXKK)3X-CONH2 (X=I, L and V) have been investigated in pure water and different concentration of 2,2,2-Trifluoroethanol (TFE) by molecular dynamics simulation. The simulations show that the helix conformation becomes more stable as the concentration of TFE increases. Using Leucine (Leu) to replace isoLeucine (Ile) does keep more alpha-helix secondary structure content at low TFE concentration, whereas there is no much difference in helical content of these two peptides when TFE is higher than 30%. The alpha-helix of Valine (Val) mutant is unstable in all TFE concentrations. Instead, Val mutant exhibits a 310-helix conformation at 50% TFE. These simulation results suggest that the stability of alpha-helix is dependent not only on hydrophobic effect but also geometric steric matching and stabilization of TFE. It is found that C-terminus of these peptides are unstable and easy to unfold, which suggests that controlling the unfolding at the C-terminus might be an important strategy to tune the helix stability of these peptides.

Key words: Antimicrobial peptides, molecular dynamics simulation, TFE.

1. Introduction

Antimicrobial peptides (AMPs) is one promising candidate for the development of medicine due to their innate immunity. Generally, AMPs have broad-spectral bactericidal activity, high killing rate and the distinctive mode of action such as targeting the bacterial cell membrane directly [1]-[3]. The antimicrobial activity and selectivity are affected by many factors, for instance, molecule net charge, amphipathicity and distribution of the hydrophobic and hydrophilic domains [4]-[8]. As one important member of AMPs, the helical antimicrobial peptides have been studied widely and the alpha-helix content has been discussed because it represents a majority of the factors above and ultimately affects the relationship between the stability of alpha-helix secondary structure and the antimicrobial activity and selectivity [9]. In our previous study, we found that use of Ile instead of Leu in helix conserved sequences (i.e. G(XXKK)nX-CONH2 series) could not only retain their selectivity against bacteria and tumor cells but also weaken their toxicity to normal mammalian cells [9]. This amazing feather is suggested to be the weaker alpha-helical forming

propensity of Ile mutant. Circular dichroism (CD) spectroscopy was adopted to assess the secondary structures of these peptides in aqueous solution and apolar environment. Leu and Ile mutants show similar helical forming propensity (unstructured in pure water and alpha-helix in 30% TFE solution). It turns to be essential to answer the question that whether use of Ile instead of Leu could weaken the helical propensity and how the helical propensity is affected by different residues. Molecular dynamics simulation technology, which could show the atom-level structural information, becomes a powerful method to investigate the microscopic molecular behaviors. In this research, we simulated three cationic amphiphilic peptides G(XXKK)nX-CONH2 (X=I, L and V) in pure water and different concentration of TFE using full-atom molecular dynamics. TFE has been proved to be useful to mimic apolar environments and induce helical formation [10], [11]. The helical propensity of different residue in helix conserved sequences was compared and the stabilization of TFE in the antimicrobial helical peptides was discussed.

2. Methods

Molecular dynamics (MD) calculations were performed using the GROMACS 4.5.5 software [12]. The peptide molecules were built with right-handed alpha-helix secondary structure and were put in rectangular simulation boxes filled with water molecules. Periodic boundary conditions were applied to the simulation boxes. The OPLS all-atom force field and the tip4p water model were used in the simulations [13], [14]. For all systems, to relax the initial configurations, the potential energy of the system was minimized by using the steepest-descent method until it converged. Bond lengths were constrained by the LINCS algorithm [15]. The electrostatic interactions were calculated using the Particle Mesh Ewald algorithm [16] with a cutoff of 1.0 nm. The cutoff radius for the Lennard-Jones interactions was set to 1.0 nm. A dielectric constant of 1 and a time step of 2 fs were used. All simulations were performed using the constant NPT ensemble. The temperature of the system was kept constant at 300 K using the Nosé-Hoover thermostat [14] with a time constant of 0.2 ps. The density of the system was adjusted according to the first constant NPT equilibration runs. The Parrinello-Rahman Method [17]-[19] with the coupling time 1.0 ps was used to implement the barostat with 1 bar.

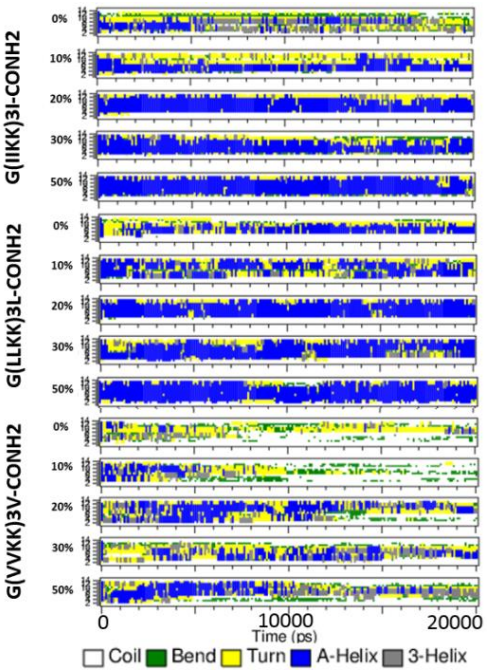

Fig. 1. Time evolution of the secondary structure of G(XXKK)3X-CONH2 (X=I, L and V) peptide in pure water and different concentrations of TFE solutions.

3. Results and Discussions

Alpha Helical Propensity of Different Residue: For all the three peptides, in pure water, the alpha-helix is rather unstable and unfolded quickly. In the case of Ile and Leu mutants, the secondary structure analysis shows that more helix content are maintained as the concentration of TFE increases. After a critical point of TFE concentration (i.e. 30% for Ile mutant), the whole backbone adopts alpha-helix conformation. Leu mutant have a lower critical concentration (20% TFE). In addition, Leu mutant keeps considerable helical content (~30%) even in pure water. This result supports directly our previous hypothesis that use of Ile instead of Leu weakens the alpha-helix propensity. The alpha-helix conformation of Val mutant is unstable in all TFE concentrations although it has more helical content at high TFE concentration. It is supposed to be due to the geometric matching effort since the Val mutant exhibits formation of 310-helix at 50% TFE, implying that Val does not favor alpha-helix conformation. The results are shown in Fig. 1.

Terminus effect: The unfolding processes are different among these three peptides. For Val mutant, the unfolding starts at any position through the sequence. Conversely, Ile and Leu mutants unfolded from the C-termini as shown in Fig. 1. This might be because the two charged LYS residues near the C-terminus are easy to be disrupted at low TFE concentration. This finding implies that the choice of residue at the C-terminus is crucial to control the stability of the alpha-helix conformation.

Confirmation of the Helical Wheel: All the three peptides are designed based on the helical wheel model. In our previous study, we have found that the separation degree between hydrophilic face and hydrophobic face has a pronounced effect on hemolysis of these peptides. It becomes important to validate whether the hydrophilic face and the hydrophobic face are as predicted. The boundary of the hydrophilic face and hydrophobic face is clear at high helical content when the charged LYS residues are almost positioned at the hydrophilic face as shown in Fig. 2. However, if the alpha-helix is unfolded, for example, in pure water, the distribution of charged LYS residue turn to be symmetrical around the center of the molecule itself as shown in Fig. 2.

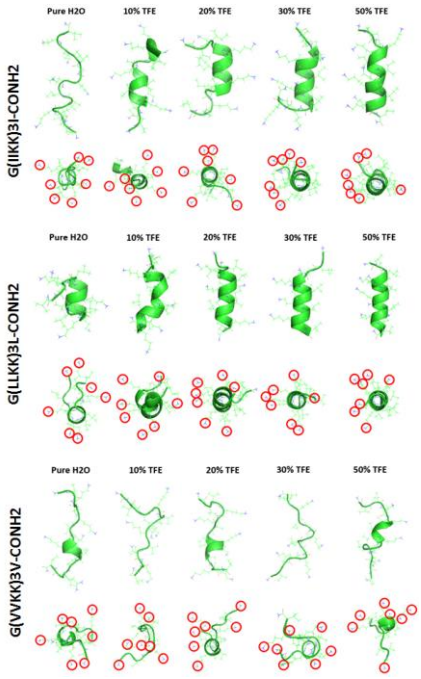

Fig. 2. Time evolution of structural snapshot of G (XXKK)3X-CONH2 (X=I, L and V) peptide in pure water and different concentrations of TFE solutions. The positions of NH3+ are highlighted by red circles.

Stabilization of alpha-helix by TFE: The previous simulation studies suggest that the stabilizing effect of TFE is due to the preferential aggregation of TFE around the helical peptides [20]. The coating displaces water and removes alternative hydrogen-bonding partners, providing a low dielectric environment which helps intrapeptide hydrogen bonds formation (Fig. 3). In our simulation, we found TFE molecules mostly locate around the hydrophobic groups of the peptides and water molecules locate close to the charged groups of the peptides in a high local concentration (Fig. 3). This result indicates that TFE solvents interact with nonpolar residues and do not disrupt the hydrophobic interactions within the peptides. The surface area analysis showed that G (XXKK)3X-CONH2 had the most hydrophilic surface area and the least hydrophobic surface area in pure water (Fig. 4). As temperature rises, the hydrophilic surface area decreases and the hydrophobic surface increases as a result of alpha-helix formation. This result indicates that the stability of TFE includes the balance of hydrophobic effect. H-bonds number analysis indicates that intra-backbone H-bonds number is positively correlated to alpha-helix content (Fig. 5(A)). In pure water, the hydrophilic backbone forms considerable amount of H-bonds with water solvents which leads an unfolding of alpha-helix. As more TFE are added, TFE could not only stabilize the hydrophbic groups but also forms H-bonds with the peptide backbone which reduces the contact between peptide backone and water solvents. (Fig. 5(B) and Fig. 5(C)).

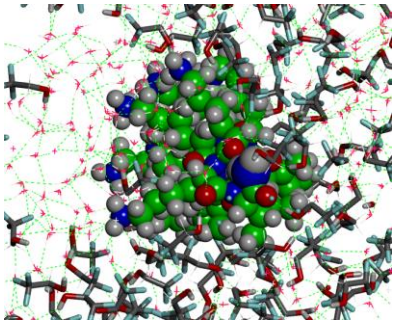

Fig. 3. Snapshots of the last frame from G(IIKK)3X-CONH2 in 30% TFE simulation at 300K. For clarity, the peptide molecule is shown in vdw model, TFE solvents are shown in stick model and water solvents are shown in line model.

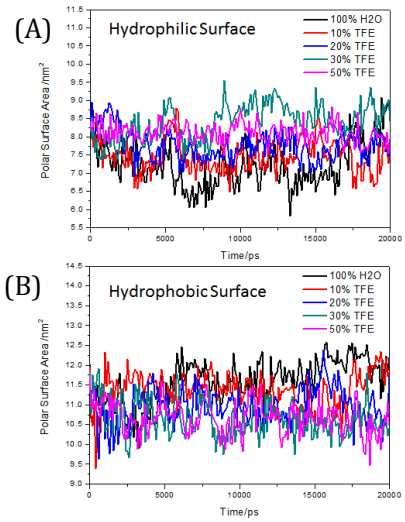

Fig. 4. Time evolution of hydrophilic surface area (A) and hydrophilic surface area (B) of G(IIKK)3X-CONH2 in pure water and different concentrations of TFE solutions.

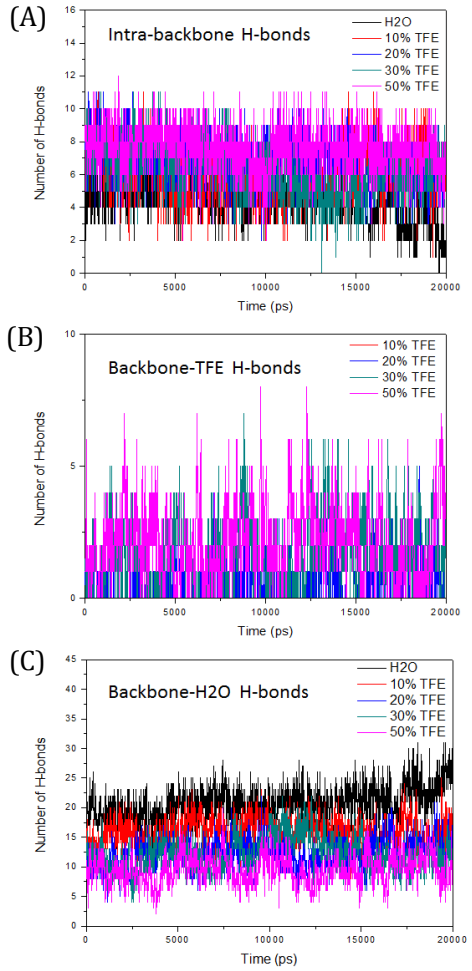

Fig. 5. Time evolution of different types of H-bonds numbers of G (IIKK)3X-CONH2 in pure water and different concentrations of TFE solutions. (A) Inter-backbone H-bonds number. (B) H-bonds number between peptide backbone and TFE solvents. (C) H-bonds number between peptide backbone and water solvents.

4. Conclusion

This research studied the alpha helix stability in different TFE concentrations. This research showed that TFE helps to stabilize the alpha-helix by stabilizing the hydrophobic sidechains and reduces the contact between peptide backbone and water solvents, providing a low dielectric environment which favors intrapeptide hydrogen bonds. This research confirmed that Ile mutant weaken the stability of alpha-helix. The reason might be the intrinsic conformation preference of the amino acid residue itself. This research also confirmed the helix wheel structure directly in specific solvent environment. This research found that C-terminus of G (XXKK)3X-CONH2 is more unstable and easy to unfold, which suggests that controlling the unfolding at the C-terminus might be an important strategy to tune the helix stability of these peptides and improve their selectivity against bacteria and tumor cells.

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