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Promoting effect of ethanol on dewetting transition in the confined region of melittin tetramer

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Abstract To study the influence of ethanol molecules on the melittin tetramer folding, we investigated the dewetting transition of the melittin tetramer immersed in pure water and 8% aqueous ethanol solution (mass fraction) by the molecular dynamics simulations. We found that the marked dewetting transitions occurred inside a nanoscale channel of the melittin tetramer both in pure water and in aqueous ethanol solution. Also, ethanol molecules promoted this dewetting transition. We attributed this promoting effect to ethanol molecules which prefer to locate at the liquid-vapor interface and decrease the liquid-vapor surface energy. The results provide insight into the effect of ethanol on the water dewetting phenomena.

Key words Dewetting transition, Melittin tetramer, Ethanol, Molecular dynamics simulation

1 Introduction

Hydrophobic interactions play an important role in many physico-chemical processes, such as protein folding[1], micelle formation[2,3], water permeation in membrane channels[4,5] and separation of hydrophobic particles[6–8]. In 1973, Stillinger F. H.[9] argued that water molecules at ambient conditions did not wet a hard wall, and formed a water-vapor interface near the wall. Later, many groups studied this phenomenon using computer simulations and theoretical analysis [10–23]. It was found that large hydrophobic solutes or surfaces can cause reorganizations of water molecules, and there is a nanoscale dewetting transition, when the hydrophobic interaction is extremely strong[24–26]. This happens when two nanoscale hydrophobic plates approach each other and reach a critical distance which is still large enough to accommodate several layers of water molecules, so the water trapped between the two hydrophobic surfaces evaporates spontaneously in a very short period of time before the collapse of the two plates. This transition occurring on a microscopic length scale is analogous to a first-order phase transition from liquid to vapor. Proteins are much more complicated than purely hydrophobic systems by exerting electrostatic forces on water[27–29]. Because of the electrostatic forces, there is no strong dewetting transition observed in the collapse of the BphC enzyme[30]. However, Berne *et al.* [13,31] studied the collapse of the melittin tetramer in water by computer simulations, and observed a marked water dewetting transition inside a nanoscale channel of the tetramer with a channel size of up to two or three water- molecule diameters.

The presence of small solute molecules, such as denaturants and alcohols, may greatly affect the hydrophobic/hydrophilic interaction in aqueous solutions, and thus may influence the thermal stability or solubility of proteins[32–34]. The mechanism of how cosolvent molecules affect hydrophobic/hydrophilic interactions is a basic issue in the protein chemistry field, but remains a matter of some controversy[23,35,36]. Thus, examining the impact of these solutes on the dewetting transition phenomenon can provide a new perspective regarding the microscopic mechanisms[36].

In order to study the influence of ethanol molecules on the melittin tetramer folding, we performed molecular dynamics simulations on the

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dewetting transition of the melittin tetramer immersed in pure water and aqueous ethanol solutions, and found marked dewetting transitions inside a nanoscale channel of the melittin tetramer in both systems. It was also found that the ethanol molecules promoted the dewetting transition. The vapor phase is preferred because ethanol molecules locate at the liquid-vapor interface, and decrease the liquid-vapor surface energy. We believe that the findings will be of help for understanding the dewetting behavior of the mixed solution in the nanoscale space, including that in the nano-devices and biosystems.

2 Simulation method

Protein melittin with a 26-residue polypeptide is a small toxic protein in honeybee venom, and often self-assembles into a tetramer, as shown in Fig.1. The starting structure of the melittin tetramer was taken from the crystal structure deposited in PDB[37]. The two dimers of the tetramer were separated by a distance D, ranging from 0.55 to 0.75 nm to create a nanoscale channel, and were solvated in pure water or 8% aqueous ethanol solution. The channel was filled with water molecules or ethanol molecules, and protein atoms were constrained with a harmonic potential with a force constant of 1000 kJ·mol^{-1}·nm^{-2}. The simulation process of the melittin tetramer was the same as the dewetting simulation used in the previous work of Berne *et al*[31]. Twenty Cl^- counterions were added to all solvated water or mixture boxes to make the system electrically neutral (Fig.1).

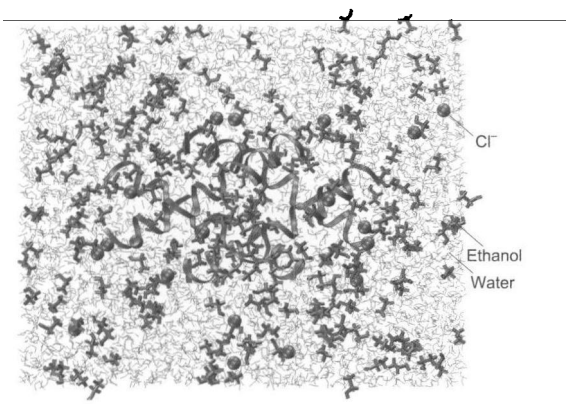

Fig.1 Schematics of imulation system. Melittin tetramer, Cl^-, water and ethanol are shown as ribbons, and van der Waals spheres, thin lines and thick bonds, respectively.

Parameters for ethanol molecules were taken from the all-atom OPLS (optimized potentials for liquid simulations) force field[38]. The rigid potential SPC/E (extended simple point charge) was used for water[39], and the SETTLE algorithm was used to keep the O–H distance fixed at 0.1 nm and the H–O–H angle at 109.47°[40]. The GROMACS[41] MD package was used for all simulations with a time step of 1 fs. Geometric combining rules were applied to calculate the Lennard–Jones interactions between different particles: $\varepsilon_{ij}=(\varepsilon_{ii}\varepsilon_{jj})^{1/2}$ and $\sigma_{ij}=(\sigma_{ii}\sigma_{jj})^{1/2}$, where ε_{ii} and σ_{ii} are the parameters of atom (i) for the Lennard–Jones diameter and well depth, respectively. The systems were run at a constant pressure and temperature (NPT) of 10^5 Pa and 298 K for 5 ns, after the energy minimization with a steepest-descent algorithm. The NPT ensemble was maintained at 298 K by the Nosé–Hoover thermostat[42] and 10^5 Pa by the Parrinello–Rahman barostat[43]. The Particle-Mesh Ewald method[44] was used to treat long-range electrostatic interactions, and van der Waals interactions were treated with a cutoff distance of 1.20 nm. The 3-ns simulations were carried out for each separation with three different initial configurations at the constant temperature and pressure using the same methods mentioned above, to explore the critical distance for dewetting D_C below which vapor is the stable phase.

3 Results and Discussion

Figure 2 shows snapshots from two 'dewetting' simulations with an initial separation distance of D= 0.55 nm. One sees from Fig.2a that water molecules inside the gap of the melittin tetramer in pure water are quickly expelled within about 100 ps. Then, a strong, cooperative water dewetting transition occurs inside the nanoscale channel in a large enough size to accommodate two or three layers of water molecules.. The entire dewetting process is about 300 ps, with a fluctuation for a short period before all the water molecules inside the channel are expelled. In Fig.2b, water molecules in the gap of melittin tetramer in the ethanol solutions are expelled, too, but it is slower than that in pure water. The ethanol molecules enter the gap of the melittin tetramer first, and then are expelled with the water molecules. In the aqueous ethanol solutions, there is also a dewetting transition, which takes about 500 ps.

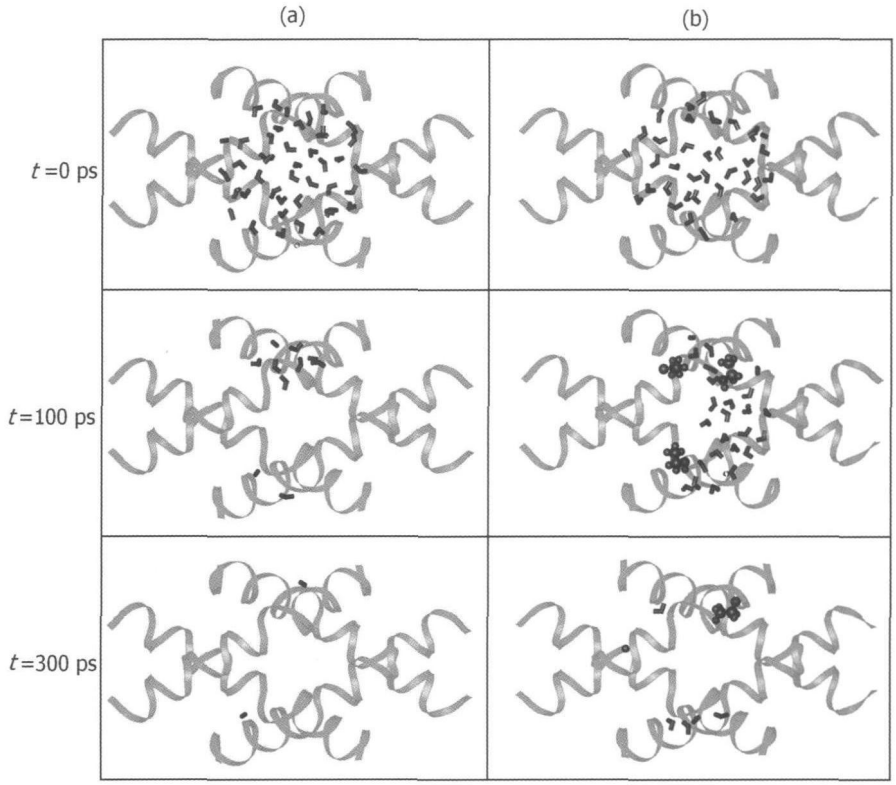

Fig.2 Snapshots of water molecules inside the gap of the melittin tetramer in pure water (a) and of water or ethanol molecules inside the gap of the melittin tetramer in aqueous ethanol solutions (b). Only water and ethanol molecules near the center of the channel were plotted, which was defined as the region with a spherical radius of 1 nm from the center of the enlarged tetramer. For clarity, the melittin tetramer, water and ethanol are shown as transparent gray ribbons, gray bonds, and van der Waals spheres, respectively.

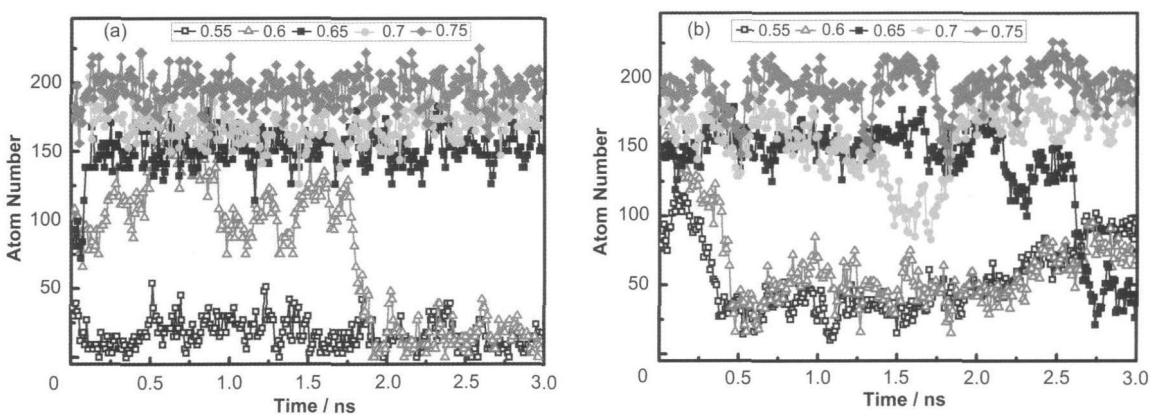

Fig.3 Time traces of the atom number inside the channel of melittin tetramer in pure water (a) and aqueous ethanol solution (b) with D=0.55, 0.6, 0.65, 0.7 and 0.75 nm.

Figure 3 plots a few time traces of the atom number inside the channel of the melittin tetramer, starting from different initial distances with one initial configuration. Fig.3a shows the results in pure water: at D=0.5 and 0.55 nm, water molecules inside the channel of the melittin tetramer are expelled and the vapor phase is dominant; at D=0.6 nm, both the liquid phase and vapor phase exist inside the channel of the melittin tetramer; at D>0.6 nm, the number of water molecules inside the channel of the melittin tetramer keeps almost equal to that with the wet initial conditions, and the liquid phase is dominant. The simulation results show that the critical distance for dewetting D_C in pure water is about 0.6 nm, which is equivalent to two water-molecule diameters. Fig.3b shows the results in aqueous ethanol solutions: water and ethanol molecules inside the channel of the melittin tetramer are expelled at D<0.65 nm, and the

vapor phase is dominant; the number of water and ethanol inside the channel of the melittin tetramer at D >0.7 nm keeps about equal to that with the wet initial conditions, and the liquid phase is dominant; the channel of the melittin tetramer vacillates between the liquid phase and vapor phase at D=0.65 or 0.7 nm. The simulation results show that the critical distance for dewetting D_C in aqueous ethanol solutions is 0.65–0.7 nm, which is larger than that in pure water. Comparing Fig.3b with Fig.3a, one finds that the melittin tetramer collapses more easily in aqueous ethanol solutions at low ethanol concentration. We note that there are many atoms inside the channel of the melittin tetramer in aqueous ethanol solutions than that in pure water.

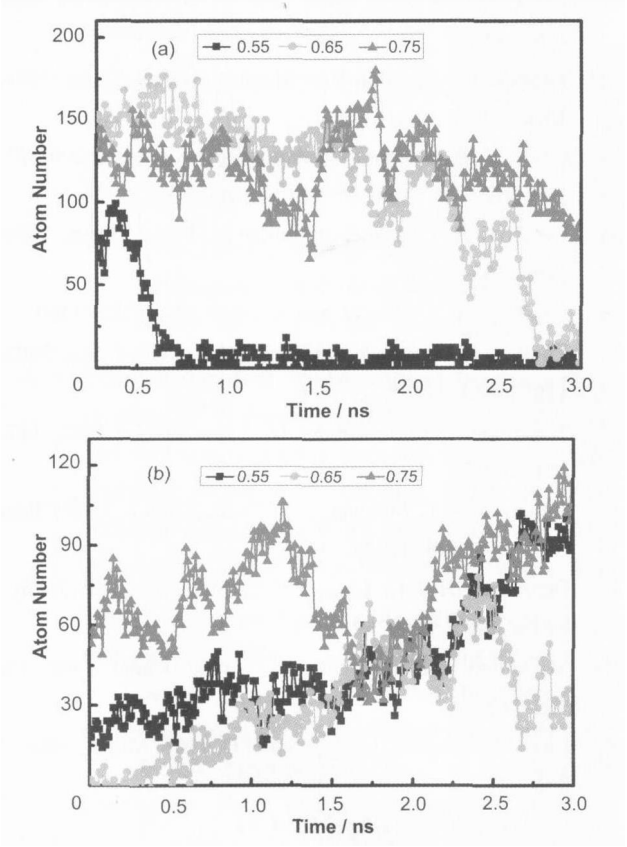

Fig.4 Time traces of the atom number of water (a) and ethanol (b) inside the channel of melittin tetramer in aqueous ethanol solutions with D=0.55, 0.65 and 0.75 nm.

To explore mechanisms of the promoting effect of ethanol on the dewetting transition, we calculated the time traces of the atom number of water and ethanol inside the channel of melittin tetramer in aqueous ethanol solutions with D=0.55 nm (in vapor phase), 0.65 nm (vacillating between two phases) and 0.75 nm (in liquid phase), respectively (Fig.4). As mentioned above, the vapor phase is dominant at D=0.55 nm, water inside the channel of melittin tetramer are expelled very quickly, with only a small fluctuation in water number, while the number of inner ethanol increase with a large fluctuation. The liquid phase is dominant at D=0.75 nm, both water and ethanol have a large fluctuation. From Fig.4b, it could be deduced that ethanol preferentially locate in the vicinity of the melittin tetramer. That is to say, ethanol molecules prefer to stay at the interfaces. Vazquez *et al.* [45] measured the surface tension of aqueous alcohol solutions at the liquid-vapor interface and found that surface tension decreases as the ethanol concentration increases for a given temperature. It can be concluded that the ethanol molecules locate at the liquid-vapor interface, and decrease the liquid-vapor surface energy through the previous results[45,46]. Then, the vapor phase is preferred in the gap of the melittin tetramer, and the critical distance for dewetting becomes larger.

4 Conclusions

The dewetting transition of the melittin tetramer immersed in 8% aqueous ethanol solutions was investigated by the molecular dynamics simulations. The obvious dewetting transitions happen inside a nanoscale channel of the melittin tetramer in pure water and aqueous ethanol solution. The dewetting process is slightly faster in pure water (~300 ps) than in aqueous ethanol solution (~500 ps). But the addition of ethanol molecules increases the critical distance for dewetting from 0.6 nm to 0.65–0.7 nm, and promotes this dewetting transition. Also, the ethanol molecules preferentially locate in the vicinity of the melittin tetramer. It could be concluded that the ethanol molecules locate at the liquid-vapor interface, and decrease the liquid-vapor surface energy. Therefore, the vapor phase is preferred in the gap of the melittin tetramer, and the critical distance for dewetting becomes larger. This observation is helpful to understanding the dewetting behavior of the cosolvent solutions.

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