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Cell-penetrating peptides (CPPs) have an ability of internalisation to inner cells through plasma membrane. The plasma membrane and lipid bilayer in experiments contain negatively charged lipids. HIV-1 Tat peptide, which is one of the CPPs, has many arginines with positive charge, and strongly interacts with negatively charged lipids. We investigate the difference between neutral lipids, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), and negatively charged lipids, 1,2-dioleoyl-*sn*-glycero-3-[phospho-rac-(1-glycerol)] (DOPG), by all-atom molecular dynamics simulations. We found that the speed of binding of Tat to lipid membrane for DOPC is more than 10 times faster than the speed for DOPG. The Tat peptides bind to the lipid membrane by attractive interaction between arginine in Tat and phosphates in lipids. Comparing the number of phosphates binding to arginine, DOPG gives a larger number than DOPC. The differences indicate the importance of negatively charged lipids for the investigation of the property of CPPs.

Keywords: cell-penetrating peptides; Tat peptide; arginine-rich peptides; lipid bilayer membrane; molecular dynamics simulations

1. Introduction

Cell-penetrating peptides (CPPs) have the ability to permeate the cell membrane and to enter living cells. Arginine-rich CPPs, such as HIV-1, Tat peptide (^{47}YGRKKRRQRR^{57}R) and oligoarginines, have high efficiency of internalisation, and have been studied for membrane-permeable vectors which work for intracellular delivery of bioactive molecules [1]. For pharmacy and medicine, it is necessary to understand the internalisation mechanism to develop drug delivery systems [2].

There are two pathways of internalisation of the arginine-rich peptides; one is endocytosis and the other is direct permeation of membrane, observed in a unilamellar vesicle [3,4]. There are various models proposed for the internalising mechanism: pore formation model [5], carpet model and inverse micelle model [6]. The pore formation was studied by molecular dynamics simulations [7–10]. The lipids used in past simulations have neutral charge, such as 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine. However, the plasma membrane and lipid bilayer in experiments contain negatively charged lipids [3,4]. Tat peptide has eight positive charges on six arginines and two lysines, and strongly interacts with the negatively charged lipids. We investigate the difference between neutral lipids, DOPC, and negatively charged lipids, 1,2-dioleoyl-*sn*-glycero-3-[phospho-rac-(1-glycerol)] (DOPG), by all-atom molecular dynamics simulations.

2. Methods

We study the binding of peptide to lipid membrane by all-atom simulations of 100 ns using GROMACS 4.0.4 [11]. The simulation systems include 128 lipids, which are DOPC (neutral lipids) or DOPG (negatively charged lipids), with five Tat peptides or without Tat, counter-ions and about 8000 water molecules, as shown in Table 1. We use periodic boundary condition and NTP ensemble. The overall temperature of water, lipids and peptides is kept constant, and each group of molecules is connected independently with the Berendsen thermostat of 323 K. The pressure is coupled to a barostat Berendsen et al. [12] at 1 atm separately in every dimension. The temperature and pressure time constants of the coupling are 0.2 and 2 ps, respectively. The average cell dimensions are estimated to be 6.5 nm in the two directions parallel to the membrane, and 10.0 nm in the direction perpendicular to the membrane. The integration of the equations of motion is performed using a leapfrog algorithm with a time step of 2 fs. This wide time step is possible using the constraint of bond length. The cut-off of 1.0 nm is applied to Lennard-Jones potential and also to the real space part of the Ewald sum of Coulomb interactions. The Fourier space part of the Ewald splitting is computed by using the particle-mesh Ewald method [13], with a grid length of 0.12 nm on the side and with a cubic spline interpolation. We use the SPC/E for water potential [14], the

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Table 1. The molecules in the simulated systems.

System	Lipid	Tat	Ion	Water
1	DOPC 128	0	0	8496
2	DOPC 128	5	Cl^- 40	8053
3	DOPG 128	0	Na^+ 128	8617
4	DOPG 128	5	Na^+ 88	8261

Notes: Systems 1 and 2 include DOPC, systems 3 and 4 include DOPG lipid and systems 1 and 3 include Tat peptides. The counter-ions are added to ensure neutrality of the system.

lipid parameters are from the works of Berger et al. [15] and the peptide parameters are from the GROMACS force field [11].

The five Tat peptides (three of them are shown in Figure 1) are placed near the lipid membrane surface as the initial states. Tat peptides occasionally diffuse to the opposite membrane surface through the water region with periodic boundary condition. We restrict Tat positions to prevent binding to the opposite membrane surface by our customised code based on GROMACS.

As the initial state of DOPC lipid membrane, we use the equilibrated coordinates (100 ns) of Siu et al. [16]. The DOPG lipid membrane is made by replacing choline of DOPC by glycerol in 1-palmitoyl-2-oleoyl-*sn*-glycero-3-[phospho-rac-(1-glycerol)] [17].

3. Results

For DOPC membrane, the snapshots of initial state and final state at 100 ns are shown in Figure 1. Tat peptides are present on the surface of DOPC membrane, and do not enter the hydrophobic core of lipid membrane. For DOPG membrane, the snapshots are similar to the snapshots of DOPC.

Time dependence of the positions of Tat peptides is shown in Figure 2(a) (100 ns) and Figure 2(b) (first 4 ns). The position is measured from the mid-surface of the membrane in the direction perpendicular to the membrane surface, as shown in Figure 2(c). We found that the speed of binding of Tat to lipid membrane from the water region for DOPC is much faster than the speed for DOPG, as shown in Figure 2. In the DOPC system, the position of Tat becomes roughly constant at about 60 ns. In contrast, in the DOPG system, the position of Tat becomes roughly constant at about 1 ns.

Tat peptides bind to the lipid membrane by attractive interaction between arginines (lysines) in Tat and phosphates in lipids. We count the binding number of phosphates per arginine and classify the numbers. When the distance between nitrogen in arginine and phosphorus is less than 0.45 nm, we count it as binding number. Figure 3 shows the ratio of the number of binding for DOPC and DOPG.

The zero binding number means that the arginines tend to go closer to water region (opposite of lipid membrane). Thirty-six per cent (30%, respectively) of arginines in Tat peptides do not contribute directly to the binding to the DOPC (DOPG, respectively) membrane. Binding numbers 1–3 mean that the arginines tend to go closer to the membrane and binding lipids. Binding number 2 for DOPG represents 30% of arginines, which is 1.8 times larger than that for DOPC. It indicates that Tat peptides interact with DOPG more strongly than with DOPC.

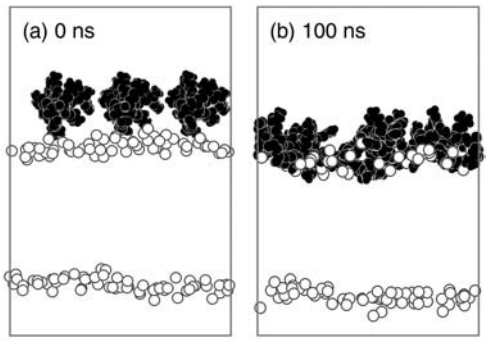

Figure 1. The snapshots at (a) 0 ns and (b) 100 ns. The black circles represent Tat peptides and white circles represent phosphorus in lipids. Other parts of lipids and water molecules are not shown.

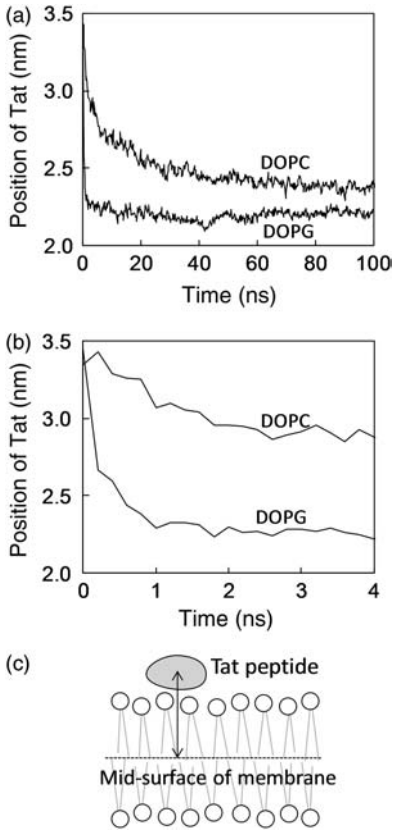

Figure 2. Time evolution of the average position of five Tat peptides in all simulation time (a) 100 ns and in (b) first 4 ns. (c) The positions are measured from the mid-surface of the membrane to the centre of mass of the peptide in the direction perpendicular to the membrane surface.

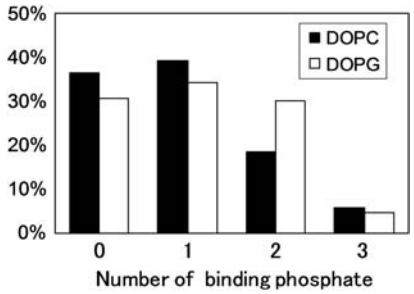

Figure 3. The number of phosphates binding to an arginine in Tat peptides for DOPC (solid bar), for DOPG (open bar). When the distance between nitrogen in arginine and phosphorus is less than 0.45 nm, we count it as binding number. We classify each arginine according to the number of phosphates bound to the arginine.

The membrane thickness of 3.9 ± 0.1 nm with Tat peptides is the same as the thickness without Tat peptides. The thickness confirms the results of Siu et al. [16] without Tat. Similarly for DOPG lipids, the thickness does not change by the addition of Tat peptides. We are performing MD simulations with coarse-grained model to obtain long-time behaviour of peptides and lipid membrane.

4. Conclusion

We investigate the difference between neutral lipids, DOPC, and negatively charged lipids, DOPG, by all-atom molecular dynamics simulations. We found that the speed of binding of Tat to lipid membrane from the water region for DOPC is more than 10 times faster than the speed for DOPG. The Tat peptides bind to the lipid membrane by attractive interaction between arginine in Tat and phosphates in lipids. The binding number of phosphates in DOPG per arginine in Tat is larger than the binding number of phosphates in DOPC. The penetration involves not only the binding to membrane, but also the deformation of the membrane and the release from the membrane after the binding. A strong binding is helpful to deform the membrane. However, if the binding is too strong, release from the membrane will be suppressed. The binding differences between DOPG and DOPC in our results indicate the importance of negatively charged lipids for investigation of the mechanism of the CPPs.

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