Chapter 29
Free Energy of Cell-Penetrating Peptide through Lipid Bilayer Membrane: Coarse-Grained Model Simulation

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Abstract Cell-penetrating peptides can permeate through the plasma membrane. The permeation ability is useful for delivery of bioactive molecules. Experiments suggest that the binding between the guanidino group in the peptide and lipid headgroups is of crucial importance in the peptide permeation through lipid membranes. We investigate the free energy profile for the permeation of the peptide through the lipid bilayer membrane with changing the binding strength by a series of coarse-grained molecular dynamics simulation. We found that the energy barrier for the permeation has the minimum at the medium strength of the binding ($\sim 2\varepsilon$). Our result suggests that the appropriate attractive interaction between peptide and lipid headgroups enhances the permeation of the peptide across the lipid membranes.

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29.1 Introduction

Arginine-rich cell-penetrating peptides (CPPs), such as HIV-1 TAT peptide (RRRKQKKRER) [1] and octaarginine (RRRRRRRR) [2], can permeate through the plasma membrane and enter the living cells with high efficiency and low toxicity [3]. The permeation ability is useful for drug delivery and gene transfection [4]. Possible pathways of the permeation include not only endocytosis but also the direct permeation through plasma membrane [2, 5]. The mechanism of the permeation of the peptide through the plasma membrane is not clear yet. The guanidino group in arginine is supposed to play an important role in the permeation of the peptide through a lipid bilayer membrane [3, 6] because guanidino group in arginine can make two hydrogen bonds to the phosphate in lipid headgroup of the plasma membrane. However, it is not clear how the binding helps the permeation. We investigate the effect of the binding on the peptide permeation by free energy calculations on the basis of molecular dynamics simulations.

The calculation of free energy profile of the penetrating molecules along the bilayer normal is of primary importance in understanding the permeability. Free energy profile of a water molecule has been successfully investigated by all-atom molecular dynamics (MD) simulations [7–13], though the evaluation of free energy profile of a large molecule such as peptide is a nontrivial task with atomic details. Especially, since CPPs induce large deformation of the lipid bilayer membrane, such as bending and inverted micelle formation [14–17], a longtime MD simulation of a larger membrane will be required for the free energy calculation. Thus, all-atom MD simulation seems too expensive to obtain well-converged free energy estimation.

Coarse-grained (CG) model can decrease the simulation time drastically by approximating the details of atomistic level. Although several different CG models are available for the lipid systems [18–21], we rather use a simple lipid model consisted of three particles, which is thought to be reasonably accurate to discuss the general feature of free energy profile for the peptide permeation. Using the simple model, we obtain an efficient computation to evaluate the free energy profile with a reduced statistical error. In this study, we especially investigate the free energy profile in relation to the binding strength of the arginine to the lipid headgroup particles.

29.2 Method

29.2.1 Coarse-Grained Model

The CG lipid molecule is represented by three particles: one hydrophilic head particle and two hydrophobic particles [17]. The three particles make a chain linked by harmonic spring bonds: $U_{\mathrm{bond}}(r_{ij}) = (K_{\mathrm{bond}}/2)(r_{ij} - \sigma)^2$. r_{ij} is the distance between ith and jth particles, and K_{bond} of $200\varepsilon/\sigma^2$ is the spring constant. ε and σ are the energy and length units used in the CG model. Angle bending potential is

Table 29.1 Lennard-Jones potential parameters

Particles		$\varepsilon_{ij}/\varepsilon$	σ_{ij}/σ
Water	Water	1.0	1.0
Water	Peptide	1.0	1.0
Water	Lipid head	1.0	1.0
Water	Lipid tail	0.3	1.2
Peptide	Peptide	1.0	1.0
Peptide	Lipid head	$\varepsilon_{\mathrm{p}}/\varepsilon$	1.0
Peptide	Lipid tail	0.3	1.2
Lipid head	Lipid head	1.0	1.05
Lipid head	Lipid tail	0.3	1.2
Lipid tail	Lipid tail	0.5	1.05

applied for three connecting neighboring particles: $U_{\mathrm{angle}}(\theta) = (K_{\mathrm{angle}}/2)(\theta - \theta_0)^2$, where K_{angle} of 1.0ε is the spring constant, θ is the angle of connected bonds, θ_0 is the constant of π, and a peptide is represented by a chain of four particles. Since CPPs are known to have random coil structure [22], the angle bending and torsion potentials are not employed for the peptide. Water is treated as a single site. All particles are assumed to have the same mass of M, which is the mass unit in the CG model. We use the Lennard-Jones (LJ) potential with the cutoff length of 2.5σ for all particles:

$$U_{LJ}(r_{ij}) = 4\varepsilon_{ij}\left(\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}}\right)^{6}\right) \tag{29.1}$$

The parameters for potential depth ε_{ij} and size of particle are adjusted to satisfy the following four required properties of lipid and water system: (1) the system should show spontaneous formation of lipid bilayer membrane from a random configuration, (2) the bilayer should be in a fluid phase, (3) the model has to produce a reasonable density profile along the bilayer normal, and (4) the model has to reproduce the experimental bending modulus of lipid membrane [16, 17]. The parameters ε_{ij} and σ_{ij} are listed in Table 29.1. The LJ parameters of $\varepsilon_{ij} = 1.0$ and $\sigma_{ij} = 1.0$ are uniformly used for hydrophilic particles except for the lipid headgroups, while the LJ parameters of $\varepsilon_{ij} = 0.3$ and $\sigma_{ij} = 1.2$ are used for the interaction between hydrophilic and hydrophobic particles. The LJ parameters of $\varepsilon_{ij} = 1.0$ and $\sigma_{ij} = 1.05$ are used for headgroups. The slightly large value of $\sigma_{ij} = 1.05$ for the lipid headgroups is needed to stabilize the lipid membrane against the bending. The interaction parameter ε_{p} is ε_{ij} between the arginine and lipid headgroup, which is changed in the range of 1.0ε to 3.0ε in a series of MD simulations. The simulation system is composed of a single peptide, 512 lipids, and 5,000 water particles. The NPT ensemble was used to simulate the system in the periodic boundary condition. The pressure was controlled to 1.0 bar ($0.015\ \varepsilon/\sigma^2$) by the Parrinello-Rahman method [23] with semi-isotropic cell fluctuation, where box sizes in x and y directions were kept the same. The normal to the lipid membrane was taken along the z direction. The box dimension along x, y, and z

directions was about 21σ, 21σ, and 19σ. The temperature was controlled at 0.6ε by the Langevin thermostat [24]. The units of CG model are obtained from three quantities. (1) The energy unit ε of 0.99 kcal/mol is obtained from temperature of 0.6ε as room temperature of 300 K. (2) The length unit σ of 0.90 nm is obtained from a comparison of the thickness of lipid bilayer membrane of 4.1σ for CG model and 3.71 nm for dioleoylphosphatidylcholine (DOPC) membrane [25]. (3) The mass unit M of 1.4×10^{-24} kg is obtained by a comparison of water density $0.86\,M/\sigma^2$ for CG model water and 1.0 g/cm^3 for water, which means that a single CG water site represents 42 water molecules.

29.2.2 Thermodynamic Integration

We calculate free energy profile $\Delta G(z)$ using thermodynamic integration. We choose the reaction coordinate as the position z of the center of mass of the peptide along the membrane normal:

$$\Delta G(z) = G(z) - G(z_{\mathrm{w}}) = \int_{z_{\mathrm{w}}}^{z} \left\langle \frac{\partial U_z}{\partial z} \right\rangle_z \mathrm{d}z \tag{29.2}$$

$$U_z = \frac{1}{2} K_{\mathrm{p}} (z_{\mathrm{p}} - z)^2 \tag{29.3}$$

$\langle \cdots \rangle_z$ is an ensemble average with the fixed point of z. z_{w} is the position in the water region far from the membrane, so that $\Delta G(z)$ is the free energy difference measured from the water region. The ensemble average is taken at each point of z over the time interval of $5 \times 10^4 \tau$ to $10^5 \tau$. $\tau = \sqrt{M/\varepsilon} = 15$ ps is the time unit of CG scale. z_{p} is the position of the peptide. K_{p} is the spring constant.

The reaction coordinate has to be determined with respect to the membrane position along the membrane normal. However, once the membrane largely deforms or bends, the center of mass of the whole membrane is not useful to determine the effective membrane position for the peptide. To prevent the uncertainty of the membrane position due to the deformation, we use the effective membrane position, z_{lip}, using the local membrane patch near the peptide, which was defined with a weight function as follows:

$$z_{\mathrm{lip}} = \frac{\sum_i z_i w(x_i - x_{\mathrm{pep}}, y_i - y_{\mathrm{pep}})}{\sum_i w(x_i - x_{\mathrm{pep}}, y_i - y_{\mathrm{pep}})} \tag{29.4}$$

The summation is taken over the lipid positions, so x_i, y_i and z_i are the coordinates of ith particle of lipid molecules, and x_{pep} and y_{pep} are the coordinates of the center of mass of the peptide. The weight function, $w(x, y)$, is defined as

$$w(x, y) = \exp\left(-\frac{(x^2 + y^2)}{R_{xy}^2} \right) \tag{29.5}$$

R_{xy} is the width of the weight function. We set $R_{xy} = 4\sigma$, which is comparable to the thickness of the membrane. The defined membrane position should be kept at the same position through the simulation time. Thus, we introduce an external potential U_{lip} to keep the membrane position to the initial position z_{lip0}:

$$U_{\mathrm{lip}} = \frac{1}{2} K_1 (z_{\mathrm{lip}} - z_{\mathrm{lip0}})^2 \tag{29.6}$$

The spring constant K_1 was set to $20\varepsilon/\sigma^2$.

29.3 Results

29.3.1 Permeation of Water

Figure 29.1a shows the density profile of each segment along the normal to the membrane. The distribution of the lipid headgroups has two peaks at $z = \pm 2\sigma$. We show free energy profile $\Delta G(z)$ for the permeation of a water particle in Fig. 29.1b. $\Delta G(z)$ has a positive value in the membrane region of $-2\sigma < z < 2\sigma$ and maximum value of 6 kcal/mol (6ε) at the center of the membrane $z = 0$. Similar results have been found in atomistic simulations [7–13]; the free energy barrier for the water permeation was found to be 6–20 kcal/mol around the center of the membrane. However, taking into account the fact that the CG water particle represents to 42 water molecules, the present CG model underestimates the free energy barrier significantly.

29.3.2 Permeation of Peptide

Figure 29.1c shows $\Delta G(z)$ for the permeation of the peptide with various potential depths ε_{p} between peptide and lipid headgroups. For $|z| > 5\sigma$, the peptide is in the water region, and $\Delta G(z)$ is almost flat. The distance of 5σ from the membrane center corresponds to the sum of peptide radius 2σ and half of the membrane thickness 3σ.

When $\varepsilon_{\mathrm{p}} = 1.0$, a high free energy barrier in the membrane region is formed. Thus, the peptide would mostly stay in the water region. For $1.5 \leq \varepsilon_{\mathrm{p}} \leq 2.5$, $\Delta G(z)$ has a local maximum around $z = 0$ and has two minima around $z = \pm 2\sigma$, as shown in Fig. 29.1c. $z = \pm 2\sigma$ are the positions of the lipid headgroups, as shown in Fig. 29.1a. It means that the peptide stays on the surface of bilayer membrane, as shown in Fig. 29.2a. In the experiments of DOPC/DPPC membrane and arginine-rich CPPs [22], CPPs stay on the surface of the lipid membrane. Thus, the parameter value of $1.5 \leq \varepsilon_{\mathrm{p}} \leq 2.5$ is supposed to provide a reasonable effective interaction to simulate the realistic arginine-rich CPPs. For $1.5 \leq \varepsilon_{\mathrm{p}} \leq 2.5$, $\Delta G(z)$ has a (local) maximum around the center of the membrane $z = 0$. At this position,

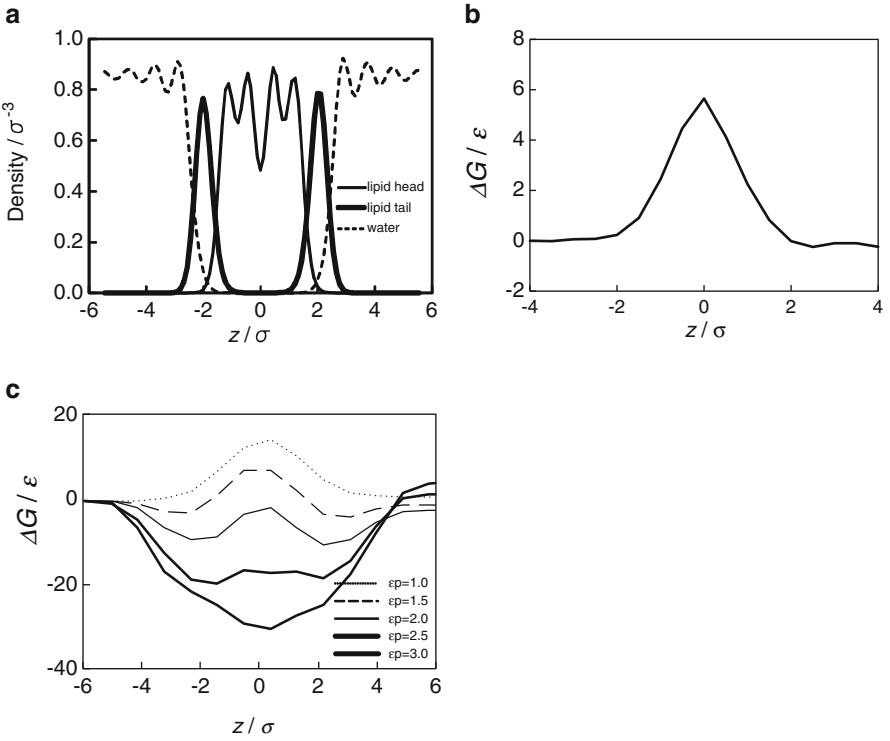

Fig. 29.1 (**a**) Particle density of lipid headgroup, lipid tail, and water molecules as a function of z. (**b**) Free energy profile $\Delta G(z)$ of water permeation. (**c**) $\Delta G(z)$ of peptide permeation with various values of ε_p. Horizontal axis z is the position of the peptide from the center of the bilayer membrane along the normal to the membrane

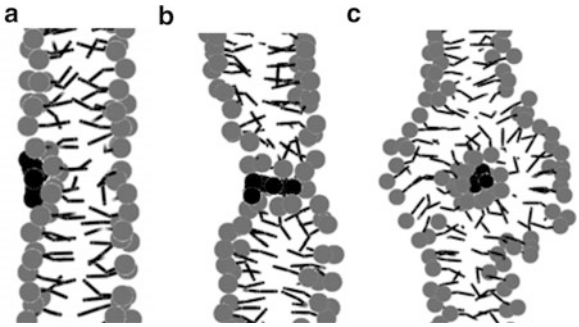

Fig. 29.2 Snapshots of MD simulations. (**a**) The peptide stays on the surface of the lipid membrane. $\varepsilon_p = 2.0$ and $z_p \sim -2\sigma$. (**b**) Transmembrane position of the peptide with $\varepsilon_p = 2.0$ and $z_p \sim 0$. (**c**) Inverted micelle formation by the peptide with $\varepsilon_p = 3.0$ and $z_p \sim 0$. Gray circles are lipid headgroups, black sticks are lipid tails, and black circles are peptides. Water molecules are not shown

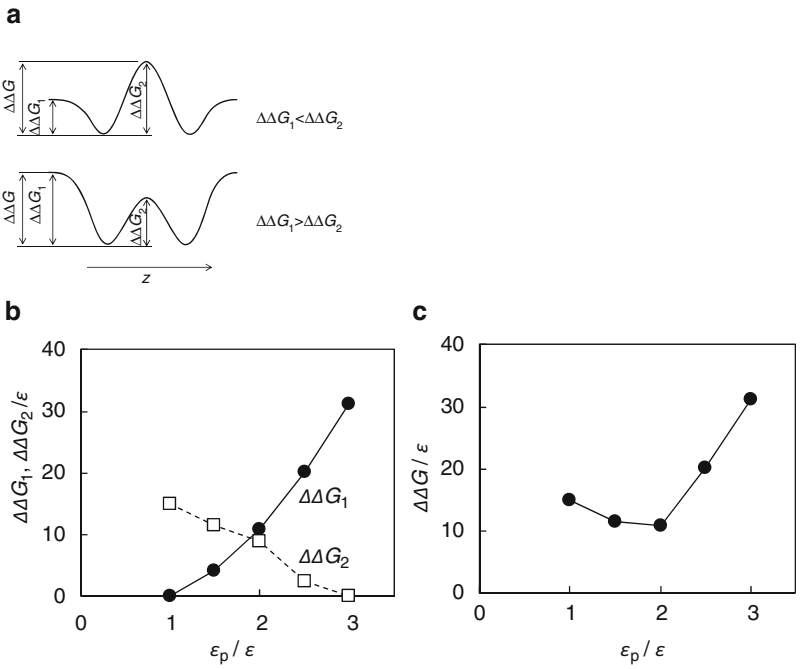

Fig. 29.3 $\Delta\Delta G$ for permeation of peptide with various values of ε_p. (**a**) Explanation of $\Delta\Delta G$, $\Delta\Delta G_1$, and $\Delta\Delta G_2$. (**b**) ε_p dependence of $\Delta\Delta G_1$ (*solid line*) and $\Delta\Delta G_2$ (*dashed line*). (**c**) ε_p dependence of $\Delta\Delta G$

for $1.5 \leq \varepsilon_p \leq 2.5$, the peptide shows transmembrane structure, as shown in Fig. 29.2b. There is a toroidal pore around the peptide. At $\varepsilon_p = 3.0$ and $z = 0$, $\Delta G(z)$ has the minimum, as shown in Fig. 29.1c, and the peptide induces an inverted micelle, as shown in Fig. 29.2c. The peptide can have a larger number of the neighboring lipid headgroups by forming the inverted micelle structure. Due to the inverted micelle formation, the binding energy between the peptide and lipid headgroups is gained at the cost of the membrane bending energy. When the binding is strong (ε_p is large), the formation of inverted micelle is advantageous with respect to the free energy.

We use the constraint potential described in Eqs. 29.2 and 29.3 for the calculation of $\Delta G(z)$ by thermodynamic integration. An MD simulation without a constraint potential has shown that the equilibrated position of the peptide agrees with the minimum of $\Delta G(z)$.

29.3.3 Free Energy Barrier

We discuss here the free energy barrier for the permeation of the peptide through the lipid bilayer. We introduce here two values $\Delta\Delta G_1$ and $\Delta\Delta G_2$. The former is

$\Delta G(z)$ at the minimum, while the latter is the difference of $\Delta G(z)$ at $z = 0$ and at the minimum (see Fig. 29.3a). $\Delta\Delta G_1$ and $\Delta\Delta G_2$ are plotted as a function of ε_p in Fig. 29.3b. With increasing ε_p, $\Delta\Delta G_1$ increases while $\Delta\Delta G_2$ decreases. $\Delta\Delta G_1$ is mainly determined by the binding strength between the peptide and the lipid headgroups. The peptide with large ε_p binds strongly to the lipid headgroups, and $\Delta\Delta G_1$ for the peptide going out of the membrane becomes large. On the other hand, $\Delta\Delta G_2$ is mainly explained by the exclusion of the hydrophilic peptide from hydrophobic region composed of lipid tails. The peptide with a large ε_p can induce the morphology change of the bilayer structure, which reduces $\Delta\Delta G_2$.

$\Delta\Delta G$ is defined as the difference between the maximum and minimum of $\Delta G(z)$, which is equal to the larger of $\Delta\Delta G_1$ and $\Delta\Delta G_2$ (see Fig. 29.3a). $\Delta\Delta G$ hits the minimum at the medium value of $\varepsilon_p = 2.0$, as shown in Fig. 29.3c. The smaller $\Delta\Delta G$ is, the easier the peptide permeates through the membrane. Our result suggests that the moderate interaction between the peptide and lipid headgroups encourages the permeation of the peptide through the lipid bilayer.

The binding energy of a single arginine residue on POPC membrane is experimentally estimated as 0.8 kcal/mol [26]. The peptide in the present CG model represents a poly-arginine chain of 21 residues. We estimate that the binding energy of 21 arginines is $0.8 \times 21 = 16.8$ kcal/mol, $\sim 17\varepsilon$. From Fig. 29.3b, the binding energy $\Delta\Delta G_1 \sim 17\varepsilon$ is obtained when ε_p is about 2.3. This value of $\varepsilon_p \sim 2.3$ calculated from the experimental binding energy is close to our estimation of $\varepsilon_p \sim 2.0$ at the minimum $\Delta\Delta G$.

It was experimentally suggested that the guanidino group, which makes two hydrogen bonds to phosphate in lipid headgroups, was needed for the peptide permeation [3]. In our CG model, we have found that the moderate attractive potential decreases the energy barrier of permeation. Our results reveal that there is an appropriate binding strength of peptide to the lipid headgroups to encourage the CPPs permeation through lipid membranes.

29.4 Conclusion

We have investigated the molecular mechanism of permeation of the CPPs through the lipid bilayer membrane using coarse-grained molecular dynamics simulations. Especially we examined the effect of the affinity of the peptide to the lipid headgroups on the permeation of the CPPs by changing the potential depth ε_p in the range of 1–3ε. We calculated the free energy profile of the peptide across the lipid bilayer with various values ofε_p using thermodynamic integration. With increasing ε_p, the position of free energy minimum is shifted from the water region to the surface of the membrane and eventually to the center of the membrane accompanied by a formation of an inverted micelle. When ε_p is small ($\sim 1\varepsilon$), the peptide is expelled from the membrane due to the high free energy barrier in the membrane region. When ε_p is large ($\sim 3\varepsilon$), the free energy barrier for the peptide to go out of the membrane is large. Thus, the free energy barrier hits the minimum at the

medium value ($\sim 2\varepsilon$) of ε_p. The result reveals that the moderate attractive interaction between the peptide and the lipid headgroups encourages the permeation of the peptide most. Our CG simulations imply the importance of the attractive interaction between peptide and lipid headgroups to explain an enhanced permeation of the hydrophilic CPPs across the lipid membrane.

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