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RESEARCH ARTICLE

Dimensions of receptor-ligand complex and the optimal radius of endocytosed virus-like particle

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Recent experiments have pointed out that cellular uptake is strongly dependent on the physical dimensions of endocytosed nanoparticles and the optimal radius of endocytosed virus-like particle coated by transferrin is around 50 nm. As the same time, the dimensions of receptor-ligand complex have strong effects on the size-dependent exclusion of proteins in cell environments. Inspired by these experimental results, a continuum elastic model is constructed to resolve the relationship between the dimensions of receptor-ligand complex and the optimal radius of endocytosed virus-like particle. These results demonstrate that the optimal radius of endocytosed virus-like particle depends on the dimensions of receptor-ligand complex and the dimension of receptor-ligand complex reduces the depletion zone.

Keywords cellular uptake, depletion effects, dimension of receptor-ligand complex, elasticity theory

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1 Introduction

Many viruses and virus-like particles enter animal cells via receptor and clathrin mediated endocytosis, but recent experimental results show that influenza virus can enter and infect cells in the absence of clathrin-mediated endocytosis [1–3]. Therefore, the current theoretical works on endocytosis only include the energy gained from ligand-receptor complex, the elastic recoil of the biomembrane and the elasticity energy of biomembrane [4–6], which encounter challenges from nano-experiments elucidating the mechanism of endocytosis. These experiments pointed out that the cellular uptake is strongly dependent on the physical dimensions of endocytosed nanoparticles, showing that the optimal radius of endocytosed virus-like particle is around 50nm [7, 8]. After analyzing the previous theoretical works, the common fact is that the dimension of the ligand-receptor complex is not considered. An experiment [9] based on quantum dots has demonstrated that the dimension of complex has strong effects on the size-dependent exclusion of proteins. Its dimensions [10, 11] can compare with the size of some small particles in cell environments. Thus, what is the relation between the optimal radius of endocytosed virus-like particle and dimensions of receptor-ligand complex emerges. For resolving the question, a continuum model based on equilibrium mechanics is proposed, which includes the effects of the dimension of ligand-receptor complex.

2 Theoretical model

As shown by Fig. 1, it is the endocytosis process of virus-like particle. On virus-like particle adhesion to biomembrane, its adherence and engulfment at the adhesion zone require specific and nonspecific binding interactions to overcome the resistive forces that hinder particle uptake.

In the continuum mechanics model, four energies are considered, i.e., (i) E_1: The favorable energy from depletion effects, which originates from entropy and is affected by the dimension of ligand-receptor complex; (ii) E_2: The favorable energy of ligand-receptor complex; which is proportional to the number of ligand-receptor complexes in the adhesion zone; (iii) E_3: The unfavorable distortion energy of the biomembrane; (iv) E_4: The unfavorable energy due to the deformation of the biomembrane. R, δ, h and r in the following equation are the radius of virus-like particle, the dimension of receptor-ligand complex, the engulfment of virus-like particle and the radius of small bioparticle in cell environments, respectively.

For the case that the dimension (δ) of the complex of ligand on the virus-like particles and receptor on biomembrane is not considered, while the virus-like particle approaching the biomembrane, a limit gap between the virus-like particle and the biomembrane exits and its length should be the diameter ($2r$) of small particles in the cell environments. The volume between the virus-like particle and biomembrane corresponding to the limit gap is the depletion volume, while the virus-like particle approaches the biomembrane more closely, until it overlaps with the biomembrane, the depletion volume disappears. Therefore, the depletion volume should be the volume difference of two spherical crowns with the radius being $R+2r$ and R, respectively.

$$\frac{2\pi h}{3R}[(R+2r)^3 - R^3] \tag{1}$$

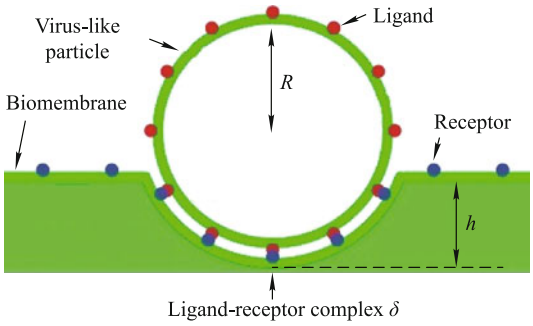

Fig. 1 Representations of endocytosis process of virus-like particle and the effects of dimension of ligand-receptor complex on the depletion effects. The green sphere is the virus-like particle and the green sheet is the biomembrane which includes cytoskeleton.The red and blue solid points represents ligand and receptor, respectively. R, δ and h are the radius of virus-like particle, the dimension of receptor-ligand complex and the engulfment of virus-like particle, respectively.

For the case the dimension of the ligand-receptor complex (δ) is considered, the virus-like particle can not approach the biomembrane infinitely, there is a gap between the virus-like particle and biomembrane and its length is the dimension of the ligand-receptor complex (δ). Compared with the case that the dimension of the complex of ligand on the virus-like particles and receptor on biomembrane is not considered, the depletion volume is reduced partially, which should be the volume difference of two spherical crowns with radius being $R+2r-\delta$ and R, respectively,

$$\frac{2\pi h}{3(R+\delta)}[(R+2r-\delta)^3 - R^3] \tag{2}$$

According to Van't Hoff relation [11], the nonspecific energy from depletion effects should be

$$E_1 = -\frac{2\pi h}{3(R+\delta)}c[(R+2r-\delta)^3 - R^3] \tag{3}$$

Here c is the concentration of small bioparticle in cell environments.

The surface ligand allows the virus-like particles to interact with receptor on biomembrane specifically. At the adhesion zone, the favorable contact energy between the ligand and the receptor is proportional to the adhesion area, the receptor – ligand binding energy f, the receptor – ligand complex density ρ at the adhesion zone, the favorable contact energy can be written as

$$E_2 = -2\pi f\rho h(R+\delta) \tag{4}$$

Ultimately, these cooperative interactions above generate sufficient thermodynamic energy to overcome the elastic recoil of the biomembrane including the bending, stretching energy and elasticity energy of biomembrane. The bending and stretching energy can be expressed by Hefrich energy [12] as the following:

$$E_3 = \frac{4\pi kh}{R+\delta} + \pi\lambda h^2 \tag{5}$$

where k and λ are bending rigidity, surface tension of the biomembrane, respectively.

At the adhesion zone, the boundary of the overlapping region between the virus-like particle and the biomembrane is treated as a circle, the virus-like particle and the biomembrane are uniform and isotropic. The cell is much larger than the virus-like particle, so the final resistive energy from the elastic energy of biomembrane can be written as [13]

$$E_4 = \frac{2\pi(R+\delta)^{0.5}h^{2.5}}{5\mu} \tag{6}$$

Here $\mu = \frac{3}{4}(\frac{1-\sigma_1^2}{\varepsilon_1} - \frac{1-\sigma_2^2}{\varepsilon_2})$, it is related to the Young's modulus and Poisson ratio of virus-like particle and biomembrane. σ_1 and ε_1 are the passion ratio and Yang's modulus of virus-like particle, respectively, σ_2 and ε_2 are those of biomembrane. The Young's modulus of the biomembrane is much less than that of virus-like particle, which makes $\frac{1-\sigma_1^2}{\varepsilon_1} \ll \frac{1-\sigma_2^2}{\varepsilon_2}$, so μ is only determined

by σ_2 and ε_2. The Young's modulus of the biomembrane is on the order of 10 kPa or less and the Poisson ratio of the biomembrane is taken to be 0.5, which make $\mu = \frac{3}{4}\frac{1-\sigma_1^2}{\varepsilon_1} = 256.25$. k_BT is taken as the unit of all the energies mentioned above, and the choice of the physical constants in the energies is guided by experimental data. The bending modulus of biomembrane is typically of the order of 10–20 and the surface tension of the biomembrane is around 0.005/nm^2 [6]. The receptor-ligand binding energy is estimated to be of the order of 10–25 [14].

3 Results and discussion

Combing all the energies mentioned above, we obtain

$$E = -2\pi f\rho h(R+\delta) - \frac{2\pi h}{3(R+\delta)}c[(R+2r-\delta)^3 - R^3] + \frac{4\pi kh}{R+\delta} + \pi\lambda h^2 + \frac{2\pi(R+\delta)^{0.5}h^{2.5}}{5\mu} \quad (7)$$

As shown by the curve groups I and II in Fig. 2, the engulfment depth h_0 at the equilibrium state is the root of the equation $\frac{\partial E}{\partial h}|_{h_0} = 0$. The total energy decreases with the concentration of small particles increasing for either of the curve groups, for the dimension of complex reduces the depletion effects. With the dimension of complex increasing from 10 nm to 40 nm, the total energy increases, which originates from the reduction of depletion volume caused by the increasing of dimension of complex. Actually, the results in Fig. 2 reflect the competition between the depletion effects and the dimension of complex in the endocytosis process.

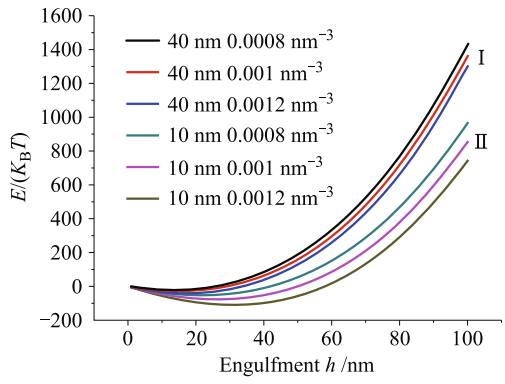

Fig. 2 Total energy of the biomembrane-virus-like particle interaction as a function of the virus-like particle engulfment depth. There are two curve groups I and II, both of which are with the same concentration change ranging from 0.0008 nm^{-3} to 0.0012 nm^{-3}, group I and group II are with different dimension of complex, corresponding to 40 nm and 10 nm, respectively.

At the equilibrium state, the relation between R and r can be identified by Eq. (8):

$$-2\pi f\rho(R+\delta) - \frac{2\pi}{3(R+\delta)}c[(R+2r-\delta)^3 - R^3] + \frac{4\pi k}{R+\delta} + 2\pi\lambda h + \frac{\pi(R+\delta)^{0.5}h^{1.5}}{\mu} = 0 \quad (8)$$

As shown in Fig. 3, the transition point corresponds to the optimum radius of virus-like particle, which depends on the dimension of complex and increases with the dimension of complex ranging from 30 nm to 44 nm. While the dimension of complex is 40 nm, the corresponding optimum radius of virus-like particle should be around 25 nm, namely, the diameter is around 50 nm, which coincides with recent experiments [7, 8]. These experiments proved that intracellular uptake of different sized transferrin-coated gold nanoparticles depends on the dimension of the gold nanoparticle. In comparison to other transferrin-coated gold nanoparticles, the one with the radius being 50 nm has the maximum uptake number per cell under the same conditions.

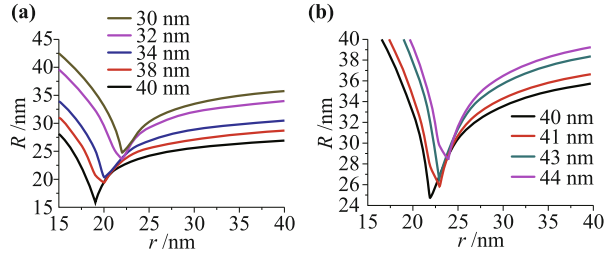

Fig. 3 Contours of dimension of ligand-receptor δ, as a function of R and r, the radii of large particles and small particles, respectively. **(a)** From bottom to top, dimension of ligand-receptor δ ranges from 30 nm to 40 nm. **(b)** Dimension of ligand-receptor δ ranges from 40 nm to 44 nm.

Comparing our theoretical results above with the experimental ones, one hypothesis that the dimension of complex determines the optimum radius of virus-like particle can be inferred, which can be proved by confirming whether the size of transferrin is around 40 nm. Recent measurement with dynamic laser light scattering [15] showed that these nanoparticles have sizes of 20.2 ± 0.2 nm for Au alone and 96.7 ± 0.2 nm for transferrin-derivatived Au-transferrin particles, so the size of transferrin should be around 38.2 nm, which is consistent with the dimension of complex ($\delta = 40$ nm).

The depletion effects have been proved by many in vitro experiments and thought to play key role in real life. But in the real process of virus-like particle being endocytosed, there are many free particles in cell environments, such as non-adsorbing polymers, proteins or dissolved ions, and their size ranges from nanometer or less to few hundreds of nanometers. For some free particles, their sizes less than the dimension of ligand-receptor

complex, they can swim freely in the gap between the virus-like particle and biomembrane. Only for free particles, their size bigger than the dimension of ligand-receptor complex, they can not enter the gap between the virus-like particle and biomembrane, the depletion effects work, but the depletion volume is reduced compared with the condition without ligand-receptor complex. So in real cell system, there is competition between the depletion effects and the dimension of ligand-receptor complex, and the depletion effects are reduced by the dimension of ligand-receptor complex.

Nanoparticles have been investigated as drug carrier for imaging and delivery therapeutic agents, to enhance drug absorption, improve bioavailability, and target agents to particular organs. But the maximum uptake number of nanoparticles is related to their size and the dimension of bioconjugates coated on the nanoparticle as experiments [16] have been pointed out. Our theoretical results show highlights into the nanoparticle design, in which, the size of nanoparticle should match with the dimension of bioconjugates such as proteins or monoclonal antibodies coated on the nanoparticle, thus the maximum uptake number of nanoparticles coated with bioconjugates can be obtained.

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