Depletion effect and biomembrane budding

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Abstract Depletion effects are well known to lead to phase separation in microsystems consisting of large and small particles with short-range repulsive interactions that act over macromolecular length scales. The equilibrium mechanics between an enveloped colloidal particle and a biomembrane caused by entropy is investigated by using a continuum model. We show that the favorable contact energy stems from entropy, which is sufficient to drive engulfment of the colloidal particle, and deformation of the biomembrane determines the resistance to the engulfment of the colloidal particle. The engulfment process depends on the ratio of the radii of the larger particle and smaller particles and the bending rigidity. The results show insights into the effects of depletion on biomembrane budding and nanoparticle transportation by a vesicle.

Keywords Depletion effects · Cell adhesion and cell mechanics · Biomembrane and vesicles · Elasticity theory

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1 Introduction

Depletion effects are well known to lead to phase separation in the bulk of colloids, which consists of both large and small particles and is dominated by short-range repulsive interactions [1–4]. For example, in binary hard-sphere mixtures, the larger particles can be attracted to flat, hard walls [5–8] and the highly charged polyelectrolyte, cytoskeletal filamentous actins and cationic lipid membranes can self-assemble into stacked three-layer tubule networks [9].

Recently, the depletion effect has been directly observed in a vesicle [10]. In that experiment, a single polystyrene particle of 0.237 μm radius was enveloped in a vesicle and observed on a focal plane under a microscope. When there is no other particle in the vesicle, this polystyrene particle diffuses freely within all the available space in the vesicle. However, when the vesicle also contains other smaller particles, this relatively large polystyrene particle is clearly attracted to the wall of the vesicle.

The reason for the larger particle to prefer the curved vesicle wall is because once it locates and is confined there, the smaller particles have more space to diffuse and the entropy of the small particles increases. Since there are more of the smaller particles in the vesicle, they dominate the total entropy. Therefore, despite the entropy of the large particle decreasing due to its confinement, the increasing entropy of the small particles increases the total entropy and eventually reduces the free energy of the whole system. This physical process also has significant biological implications [11, 12]. For example, if the larger particle is replaced by macromolecules and the smaller ones by polymer coils or small globular proteins, one can see that the presence of the smaller objects will likely drive the macromolecules to aggregate to the vesicle wall. This phenomenon may be used to promote specific recognition between macromolecules such as proteins. Under another circumstance, one can imagine that as the number of smaller particles in the vesicle increases, the larger particle, due to depletion effects, will be increasingly pushed to the vesicle wall and eventually be spontaneously enveloped by the vesicle. This process resembles that of biomembrane budding during endocytosis but has been largely overlooked in previous studies of biomembrane budding [13–16].

In order to understand the depletion effects in causing a biomembrane to engulf a particle, here we introduce a continuum model based on equilibrium mechanics between the biomembrane and the particle. This model mainly considers the elastic energy of biomembrane deformation and free energy reduction when a larger particle is engulfed by the biomembrane. Our results show that the ratio of the radii of the larger particle and smaller particles and the bending rigidity of the biomembrane has important effects on the process of the larger particles being engulfed. Especially, some insights will be shown into the effects of depletion on biomembrane budding.

2 Theoretical model

When a particle is engulfed by a biomembrane, there are three contributors to the total energy cost. The first is the free energy reduction, E_1 , favoring the overlapping complex formation. The second and third, E_2 , and E_3 are the unfavorable energies due to the distortion and deformation of the biomembrane, respectively.

It is well known that depletion effects are essentially of entropic origin, which is proportional to the concentration of small particles and the volume of the depletion area.

The free energy reduction is described as, $E_1 = \int PdV$, where P is the osmotic pressure due to the concentration of small particles [17]. Both are connected by the Van't Hoff relation $P = ck_B T$, where c is the concentration of the small particles [18]. During the whole process of engulfment, c remains almost unchanged. Thus, the free energy reduction $E_1 = \int PdV$ can be expressed as

$$E_1 = -\frac{2\pi h}{3R} ck_B T[(R+2r)^3 - R^3] \quad (1)$$

where r and R are the radii of the small and larger particles, respectively, and k_B is the Boltzmann constant. While the engulfment occurs, the vesicle is bent and stretched. Thus, the second part of the energy can be expressed as the model proposed by Helfrich [19–21],

$$E_2 = \frac{k_c}{2} \oint (2H + C_0)^2 dA + \oint \lambda dA \quad (2)$$

where k_c , H and λ are the bending rigidity, the mean curvature, and surface tension of the biomembrane, respectively, for typical cells, $\lambda \approx \frac{0.005k_B T}{nm^2}$ and $k_c \approx 10k_B T$ [22–24]. C_0 is the spontaneous curvature; if the effect of the biomembrane or its environment is symmetric, C_0 can be neglected. dA is the variation of the surface element. Since the topological class of the vesicle is without change, the Gaussian curvature is not taken into account. Here, the mean curvature is $H = \frac{1}{2R}$ and the energy difference before and after the engulfment can be approximately expressed as

$$E_2 \approx 4\pi R^2 \left[\frac{k_c h}{4R^3} + \lambda \left(\frac{h}{2R} - \frac{2Rh - h^2}{4R^2} \right) \right] \equiv \frac{\pi k_c h}{R} + \pi \lambda h^2. \quad (3)$$

The final contribution to the energy of engulfment is the elastic energy of the biomembrane. Recent experimental and theoretical work [25–28] demonstrated that the biomembrane can be regarded as an elastic solid whose stability is related to its Young's modulus and Poisson ratio, which are about 10 kPa and $\frac{1}{2}$. Based on the classic theory of elasticity, the boundary of the overlapping region between the large particle and the biomembrane is treated as a circle, while the large particle and the biomembrane are uniform and isotropic. The vesicle is much larger than the big particle and the third part is [29]

$$E_3 = \frac{2\sqrt{R}}{5\varsigma} h^{\frac{5}{2}} \quad (4)$$

where $\varsigma = \frac{3}{4} \left(\frac{1-\sigma_1^2}{v_1} + \frac{1-\sigma_2^2}{v_2} \right)$, v_1 and v_2 are the Young's moduli of the biomembrane and the big particle and σ_1 and σ_2 are the Poisson ratios of the biomembrane and the big particle. Thus, the total energy E of the system can be obtained giving,

$$E = -\frac{2\pi h}{3R} ck_B T[(R+2r)^3 - R^3] + \frac{\pi k_c h}{R} + \pi \lambda h^2 + \frac{2\sqrt{R}}{5\varsigma} h^{\frac{5}{2}}. \quad (5)$$

3 Results

From the total energy, three important parameters can be derived. The engulfment depth h_0 at equilibrium state is the root of the equation $\frac{\partial E}{\partial h}|_{h_0} = 0$. Just as Fig. 1a, it indicates that h_0 is proportional to the concentration of small particles c . So it is possible for the big particle to be engulfed totally by the biomembrane as shown in Fig. 1b.

Fig. 1 **a** The total energy E of the system as a function of colloidal particle engulfment depth, the minimum of the curves provides the equilibrium engulfment depth. **b** The equilibrium engulfment depth h_0 is the function of concentration of small particles c . Obviously, at $c = 4 \times 10^{-3} \text{ nm}^{-3}$, the polystyrene particle is completely engulfed

The force driving the big particle and biomembrane is $f = -\frac{\partial E}{\partial h}$. At a fixed engulfment depth, the radius of the big particle decreases with the concentration of small particles increasing and the driving force between the big particle and biomembrane increases, as shown in Fig. 2. Both indicate that the big particle is prone to be completely engulfed with the assistance of the driving force.

In the total energy, the free energy reduction favors the engulfment and the other parts disfavor the engulfment. At equilibrium states, the critical ratio between R and r , the radii of the larger particle and smaller particles, respectively, can be determined, as shown in Fig. 3a. At a fixed concentration of small particles, the ratio between R and r has two obvious transition states. Since R is larger than r , only the first transition state, corresponding to the critical ratio $\alpha = \frac{R}{r}$, is considered. The critical ratio $\alpha = \frac{R}{r}$ depends on the concentration of small particles and increases as the concentration of small particles decreases. Dinsmore et al. predicted [10] that when the ratio of the radius of the large particles over that of the small particles, $\alpha = \frac{R}{r}$, becomes greater than 18, engulfment is favored. We included this range of particle sizes in the calculations as a special case. The critical ratio depends on the bending rigidity of the biomembrane, as shown in Fig. 3b, which corresponds to the case

Fig. 2 **a** Contours of equilibrium engulfment depth, h_0 , as a function of R with the radius of the larger particle and concentration c of small particles. **b** The force driving the engulfment; the case with h_0 , R and r being 80 nm, 100 nm and 5.6 nm

Fig. 3 **a** Contours of concentration c of small particles, as a function of R and r , the radii of large particles and small particles, respectively. From top to bottom, $h_0 = 30, 36, 40$ nm; **b** the effects of biomembrane bending rigidity; the concentration of small particles is $0.0003/\text{nm}^3$

when the concentration is $0.0003/\text{nm}^3$. The transition states appear first and then disappear with the bending rigidity increasing gradually from $1 k_B T$ to $15 k_B T$, only the intermediate bending rigidity can cause an obvious transition state. This dependence on the bending rigidity of the biomembrane is consistent with a recent theoretical investigation of the process of a soft elastic vesicle being wrapped adhesively by the biomembrane; the wrapping phases strongly depend on the bending rigidity ratio between the vesicle and the membrane [30].

4 Discussion

As for our results mentioned above, the big colloidal particle is treated as a rigid one, but from $\zeta = \frac{3}{4} \left(\frac{1-\sigma_1^2}{\nu_1} + \frac{1-\sigma_2^2}{\nu_2} \right)$, the total energy and the three parameters mentioned above depend on Young's moduli of the biomembrane and big colloidal particle ν_1 and ν_2 and the Poisson ratios of the biomembrane and big colloidal particle σ_1 and σ_2 . Thus, a critical question emerges as to whether it is reasonable to treat the big colloidal particle as rigid.

A recent single-molecule experiment [31–34] demonstrated that the regulation of mechanical properties in some biological processes has a significant biological importance. For example, for enhancing their infectivity, the human immunodeficiency virus (HIV) and other retrovirus particles undergo a maturation process, during which they experience a significant internal morphological reorganization, changing from roughly spherically symmetric immature particles with a thick protein shell to mature particles with a thin protein shell and conical core. Using nanoindentation experiments conducted by an atomic force microscope, Rouso and his coworkers [31, 32] reported that immature particles (3.16 N/m) are more than 14-fold stiffer than mature particles (0.22 N/m) and that this large difference is primarily mediated by the HIV envelope cytoplasmic tail domain. Their finite element simulation showed that for immature virions, the average Young's modulus drops more than twofold when the virus is mature (930 vs. 440 MPa). These results show that HIV regulates its mechanical properties at different stages of its life cycle, being stiff during viral budding versus being soft during entry and that this regulation is important for efficient infectivity. As for our theoretical treatment, the Young's modulus of the big colloidal particle is thought to be far bigger than the biomembrane's; the Poisson ratio is less

than 1, which makes $\frac{1-\sigma_1^2}{v_1} \gg \frac{1-\sigma_2^2}{v_2}$, thus, $\frac{1-\sigma_2^2}{v_2}$ can be neglected. The total energy and three parameters mentioned above are only related to the Young's modulus of the biomembrane v_1 (as shown in Fig. 3b) and the Poisson ratio of the biomembrane σ_1 . This case is actually consistent with the immature HIV virion budding process.

Finally, as indicated by Figs. 2a and 3a, both the radius R of the bigger particle and the critical ratio $\alpha = \frac{R}{r}$ have dependence on the concentration of small particles, which will help choose the size of nanoparticles in nano- and micro-scale experiments of their transport by a vesicle. For the virus invasion process, it is not enough to consider the depletion effect only [35], as the length of the ligand–receptor complexes ranges from 10 nm to 42 nm and the length scale is comparable to the size of some particles in cells [36], which will reduce the depletion area. So the depletion effect and the ligand–receptor interaction should be considered together in future work.

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