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Endocytosis of PEGylated nanoparticles accompanied by structural and free energy changes of the grafted polyethylene glycol

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ABSTRACT

Nanoparticles (NPs) are in use to efficiently deliver drug molecules into diseased cells. The surfaces of NPs are usually grafted with polyethylene glycol (PEG) polymers, during so-called PEGylation, to improve water solubility, avoid aggregation, and prevent opsonization during blood circulation. The interplay between grafting density σ_p and grafted PEG polymerization degree N makes cellular uptake of PEGylated NPs distinct from that of bare NPs. To understand the role played by grafted PEG polymers, we study the endocytosis of 8 nm sized PEGylated NPs with different σ_p and N through large scale dissipative particle dynamics (DPD) simulations. The free energy change $F_{polymer}$ of grafted PEG polymers, before and after endocytosis, is identified to have an effect which is comparable to, or even larger than, the bending energy of the membrane during endocytosis. Based on self-consistent field theory $F_{polymer}$ is found to be dependent on both σ_p and N. By incorporating $F_{polymer}$, the critical ligand-receptor binding strength for PEGylated NPs to be internalized can be correctly predicted by a simple analytical equation. Without considering $F_{polymer}$, it turns out impossible to predict whether the PEGylated NPs will be delivered into the diseased cells. These simulation results and theoretical analysis not only provide new insights into the endocytosis process of PEGylated NPs, but also shed light on the underlying physical mechanisms, which can be utilized for designing efficient PEGylated NP-based therapeutic carriers with improved cellular targeting and uptake.

1. Introduction

Nanoparticles (NPs) have demonstrated promising properties as therapeutic carriers which can efficiently deliver drug molecules into diseased cells to treat numerous physiological disorders [1,2]. In the NP-mediated drug delivery process, one of the most important steps is the internalization of NPs, called 'endocytosis' [3–7]. Endocytosis is an energetically driven process by which the NPs are enveloped by the lipid bilayer of the cell membrane. Cells import and export selected extracellular molecules, as well as NPs, through endocytosis and exocytosis, respectively. Depending on the cell type, the internalization mechanism and the properties of the NPs, the endocytosis process can involve a few different pathways: phagocytosis, pinocytosis, and clathrin-dependent and independent receptor-mediated endocytosis [3,4,6]. Among them, receptor-mediated endocytosis is the most efficient pathway for cellular uptake of NPs. In this process, the surfaces of NPs are coated by ligands that recognize and bind to the cell-surface receptors. If the particle is too small, the ligand-receptor binding strength is too weak to overcome the energy barrier created by bending of the cell membrane [8,9], and the NP cannot be fully internalized. On the other hand, when the particle is too large, the cellular uptake of NPs is prohibited as the receptors expressed over the cell membrane have to diffuse to the site of NP invagination [8]. Therefore, endocytosis may not occur at all or may possibly develop over a long time period, limited by the diffusivity of receptors [8,10]. Experimental studies reveal that the optimal NP diameter for receptor-mediated endocytosis is about 25–50 nm [11,12], which agrees well with theoretical predictions [8–10,13,14] and computer simulations [15–17]. Subsequently, the shape, surface charge, and stiffness of NPs were found to play specific roles in endocytosis [3–6,18–22].

In the design of the first-generation (i.e. non-PEGylated) NPs, particles with different sizes, shapes and surface charges were

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synthesized. These features offer extra degrees of freedom over the freely administrated drug molecules for potentially improving their accumulation at the diseased sites; the importance of NP size and surface charge has been well established through experiments [11,12,23]. However, most of these experimental studies operated with serum-free media or did not consider the serum-protein interactions with NPs. In vivo data show that these first-generation NPs are rapidly cleared after injection [24]. Moreover, the NPs are unstable and usually internalized by the immune cells (macrophages) during circulation. To overcome these problems, surfaces of second-generation NPs were grafted with polymer such as polyethylene glycol (PEG), which is known to be hydrophilic and biocompatible. With the help of PEGylation, the second-generation NPs demonstrated improved stability and targeting in biological systems. At the same time, the properties of these NPs were dramatically different. Due to the PEG-mediated shielding of the NP surface charge PEGylated NPs can be well dispersed in solution. More importantly, a 'stealth' shell is formed by the grafted PEG polymer that tends to prevent clearance by the immune system (opsonization) [25]. PEGylated NPs therefore display prolonged blood circulation time. Furthermore, the cellular uptake of PEGylated NPs can be reduced due to the steric interactions between grafted chains and the cell membrane [19].

To improve the endocytosis of PEGylated NPs, all the free ends of grafted PEG polymers are typically conjugated with targeting moieties such as cell-penetrating peptides (CPPs) [26–28], arginylglycylaspartic acid (RGD) peptides [29,30], or anti-HER2 antibodies [19]. With the help of these specific ligand-receptor interactions, the cellular uptake of PEGylated NPs is tremendously enhanced. Like their ungrafted counterparts, PEGylated NPs display size-dependent endocytosis, but the size dependence is more complex. Oh et al. found that PEGylated gold (Au) NPs with $d = 2.4$ nm core diameters can be delivered into the nucleus, while NPs with $d = 5.5$ nm and $d = 8.2$ nm can only be partially delivered into the cytoplasm of a model COS-1 cell line [28]. For NPs with $d > 16$ nm, cellular uptake is prohibited and NPs are found at the cellular periphery [28]. However, in other experiments, the PEGylated Au NPs with $d = 15 - 50$ nm were efficiently internalized by cancer cells [19,30,31]. Therefore, there exists a critical question: *What are the design criteria for PEGylated NPs to be quickly accepted by diseased cells?*

In the design of PEGylated NPs, there are two basic design parameters for grafted PEG polymers: one is the number of monomers per chain, N; the other is grafting density σ_p. These two parameters govern the surface morphology of PEGylated NPs as well their performance in the drug delivery process. However, experimental studies reported a varying range of these two parameters. For example, the typical molecular weight of grafted PEG polymer is about 550–5000 Da, corresponding to $N = 12$–112 [26–30,32,33]. The reported grafting densities of PEG polymers are within the range $\sigma_p = 0.2 - 2.0$ chains/nm^2 [26–30,32,33]. It is therefore not surprising that different experiments report seemingly contradictory findings for internalization behaviors of PEGylated NPs, as they use different combinations of N and σ_p.

Although the above-mentioned experiments have uncovered critical information about large scale interactions between PEGylated NPs and cells, many atomic-level questions remain to be answered, as they cannot be easily addressed by experiments. For instance, the conformational and structural properties of PEGylated NPs and their effects on drug delivery efficacy are difficult to be resolved by experiments. To understand the role of grafted PEG polymers during endocytosis, and in particular the effects of N and σ_p, we have performed large scale dissipative particle dynamics (DPD) simulations on internalization of PEGylated NPs. Existing studies primarily focused on first-generation NPs, eventually augmented with targeting moieties [8–10,13,15–17]. Our simulation results reveal that the endocytosis of PEGylated NPs heavily depends on both N and σ_p. Especially, the free energy change of grafted PEG polymer can play an important role during the endocytosis process, which apparently remained unnoticed in previous studies.

Section 2 describes the model and methods to simulate the endocytosis process of PEGylated NPs as well as the self-consistent field (SCF) theory to quantify the free energy change of PEG polymers. Section 3 contains the results from our DPD simulations and SCF analysis, and illustrates how the endocytosis process can be affected by the N and σ_p. Section 4 discusses the findings of the current work and compares them with experimental observations. Conclusions are drawn in Section 5.

2. Model and methodology

2.1. DPD simulation details

The coarse-grained molecular dynamics simulations adopted in this work are based on the DPD technique, a Lagrangian method developed for mesoscale simulations with hydrodynamic interactions. DPD has been successfully and widely used to study the behavior of biomembranes [20,22,34–36]. To model a large piece of lipid bilayer efficiently, we adopt the lipid model developed by Groot and Rabone [34]. In this model, the lipid molecule is represented by the $H_3(T_5)_2$ model (Supporting Information (SI) Sections 1.1, 1.2 and Fig. S1), where H and T denote the hydrophilic lipid heads and hydrophobic lipid tails, respectively. In the snapshots, the hydrophilic heads and hydrophobic tails are represented by the ice-blue beads and silver lines, respectively. The bending modulus and viscosity of the self-assembled lipid bilayer are found to agree reasonably well with experimental measurements [34]. Moreover, such a model has been successfully applied to study the translocation and endocytosis of different NPs [16,20–22,35,37]. In modeling the lipid bilayer, we assume that there are ~50% lipids coated with receptors to accelerate the DPD simulations, which has been proven to be a useful estimate in previous works [15,20,21]. The heads of these lipids attract the targeting moieties conjugated at the free ends of grafted chains, as described in SI Sections 1.3 and 1.4. These lipids are randomly distributed within the membrane. All the DPD simulations are performed in the NVT ensemble with time step $\Delta t = 0.01$ (dimensionless Lennard-Jones unit).

To maintain the zero lateral tension of the lipid bilayer during the DPD simulation, we adopted the N-varied DPD method to mimic a real cell membrane with large area-to-volume ratio [38]. In the N-varied DPD method, the lateral tension is maintained by monitoring the lipid number per area (LNPA) of the membrane, instead of the lateral force/pressure. The boundary region of the lipid membrane behaves like a reservoir of lipids in the DPD simulations. The LNPA of the boundary region is kept constant by adding/deleting lipid molecules. Simultaneously, the corresponding number of solvent beads is deleted/added to maintain the particle density of the whole system. Note that LNPA is directly related to the area per lipid. Thus, the N-varied DPD method offers an easy way to control lateral tension of membrane and supplies excess membrane area to release the tension induced by the internalization of PEGylated NPs. The N-varied DPD method has been successfully adopted to study the budding behaviors of multicomponent membranes [38], internalization of ligand-coated rigid NPs [39,40], as well dendrimer-like soft NPs [37]. More details are given in SI Section 1.5.

The core of our PEGylated NP is formed by 1566 close-packed 'beads' arranged on a FCC lattice circumscribed by a sphere of diameter $d = 8$ nm. The lattice constant is $\alpha \simeq 0.90$ nm. The close

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