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Computer simulation of the role of protein corona in cellular delivery of nanoparticles

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ABSTRACT

Understanding the role of serum protein in the process of nanoparticle delivery is of great importance in biomedicine. Here, by using dissipative particle dynamics simulations, we systematically investigate the interactions between the nanoparticle-protein corona complex and cell membranes of different types. It is found that the human serum albumin (HSA) will just adsorb onto charged (especially for positively charged) and hydrophobic nanoparticle surface. More importantly, we also provide specific insights into the effect of HSA adsorption on the *in vivo* transportation of nanoparticle (i.e., immune response and targeted cellular uptake). Our results show that the protein corona can change the interaction modes of hydrophobic nanoparticles and enhance the interaction of charged nanoparticles with macrophage cell membranes, while it may also cause the failure of insertion of hydrophobic nanoparticles and the loss of targeting specificity of charged nanoparticles with cancer cell membranes. These results can help better understand the biological significance of protein corona and may give some useful suggestions on better design of future nanoparticles in drug delivery.

1. Introduction

Nowadays, nanoparticles (NPs) have been widely used in biomedicine and biotechnology, for example, they can be proposed as carriers to translocate DNA (or RNA), drug molecules and other useful materials into cell interiors [1,2]. A better understanding of the interactions between nanomaterials and cells is of central importance for their potential medical applications [3–5]. Moreover, it has been realized that the nanoparticles will interact with different types of proteins when they are introduced into a biological environment (e.g., blood) [6,7]. The proteins may bind to the surface of nanoparticles and form a biological coating around the nanoparticle, which is known as the protein corona [8,9]. The protein corona can alter the physicochemical properties (e.g., size, rigidity, and surface chemistry) of nanoparticles and then lead to a new biological identity that is distinct from its synthetic identity [8–10].

Though there is no doubt that protein corona will affect the biobehaviors of nanoparticle in cellular delivery (including extracellular and intracellular delivery) [11,12], the exact role of protein corona in cellular uptake efficiency (i.e., for different nanoparticles and cell systems, the uptake levels are very different in the presence and absence of proteins [13,14]) is still largely unknown. In fact, since there exist different kinds of cells in biosystems (e.g., the macrophage cell lines to clear nanoparticles in immune system, and the cancer cell lines for nanoparticle to deliver) that nanoparticle may interact during the whole delivery process [15,16], it is of great importance for real applications to investigate the underlying mechanism of the interaction between nanoparticle-protein corona complex and cells in details.

Recently, more and more attentions have been paid to these problems and indeed there are great progresses due to the hard efforts made by many experimental researchers [11–14,17–21]. However, it is still difficult to systematically probe and visualize these processes under various conditions, due to available experimental technologies. Computer simulation, on the contrary, may offer some useful information for the molecular mechanism of these problems because it can provide the insights from the microscopic or mesoscopic view and the simulation conditions can be well controlled [22,23]. Actually, computer simulations have been widely used to study the effect of physicochemical properties of nanoparticle itself and the coating ligands on the cellular uptake of nanomaterials [24–35]. But to the best of our knowledge, the

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situations in those simulations are all in the absence of proteins, thus there may exist a big gap between the computational results and the real biological process.

In this work, we undergo the computational study to investigate the effect of protein adsorption on the cellular delivery of nanoparticles by using dissipative particle dynamics (DPD) simulations [36,37]. We will use four classical types of nanoparticles (i.e., hydrophobic, hydrophilic, negatively charged and positively charged ones) to illustrate the effect of surface properties of nanoparticle on protein adsorption. Besides, we will use two modeling membranes to simulate the macrophage cell and the targeted cancer cell membranes. As we will show below, the protein adsorption could strongly affect the bio-behaviors of nanoparticles in the cellular delivery (i.e., uptake efficiency of different cell lines). Finally, we will give the summary of the exact role of protein corona in the interaction of nanoparticles with different types of cells.

2. Model and methods

Fig. 1 shows the coarse-grain models of different components in our simulations. The nanoparticle is fabricated by arranging DPD beads (P) on a fcc lattice with lattice constant $\alpha = 0.40$nm into a desired geometry shape and volume, and all beads comprising a nanoparticle move as a rigid body [25]. The type of beads (i.e., hydrophobic, hydrophilic, and negatively or positively charged) on nanoparticle surface may be varied, which is determined by the nanoparticle properties used in the simulations.

The protein used in this work is human serum albumin (HSA), which is the most abundant protein in human blood plasma and constitutes about half of the blood serum protein [38]. HSA is a polypeptide chain of 585 amino acid residues (over 9200 atoms), and it has a molecular mass of about 67 kDa [39]. Though it is now feasible to simulate one or several HSA proteins in water by using all-atom force field, it is still difficult to include the HSA, NP, and cell membrane in all-atom simulations because the length scale is about 100 nm (several million atoms) and the time scale is at least 10 μs (several billion time steps), which is far beyond the present computing ability. Therefore, here we use a coarse-grain model for the HSA protein, which has been used in some other coarse-grain simulations (or theories) [40,41]. Each amino acid is represented by a single bead, and the beads are connected by harmonic bonds into a freely jointed linear chain. The bead type is determined by the amino acid residue. And for the simplicity, we divide the twenty types of amino acids into four types, i.e., hydrophobic (Leu, Met, Ala, Phe, Pro, Trp, Val, Ile), hydrophilic (Gly, Ser, Asn, Gln, Thr), negative (Tyr, Cys, Asp, Glu) and positive one (Lys, Arg, His) [42]. The bead sequence is in line with amino acid sequence of HSA protein (PDB file:1AO6) [39]. The secondary structures are not taken into consideration by the present model, yet we consider that such a simple protein model should still describe the main coarse-grained features of the HSA protein since it catches the hydrophobic and electrostatic interactions [15,40].

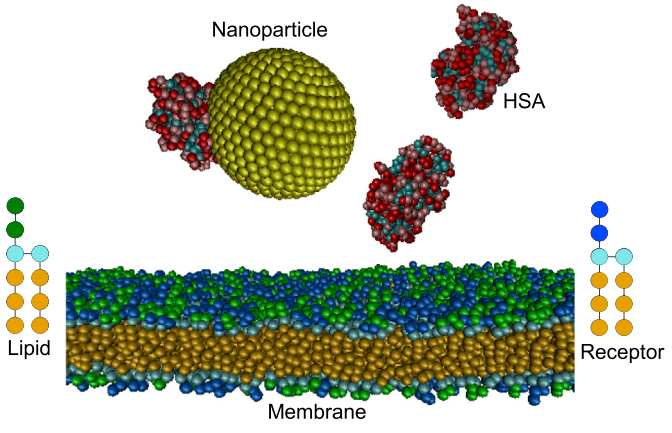

Fig. 1. Schematic illustration of the models (nanoparticles, proteins and cell membranes) in the simulations. The nanoparticle bead is yellow, the red, cyan, pink beads represents the charged, hydrophilic, hydrophobic residues in HSA protein. For cell membranes, green bead represents charged head in lipid molecule (the first green bead containing $+e$ and the second one containing $-e$), while lime bead stands for lipid head with no charge; the orange bead represents lipid tail, the ice blue bead stands for receptor head bead. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Each amphiphilic lipid consists of a headgroup containing four connected hydrophilic beads (H) and two tails with respective three hydrophobic beads (T) (Fig. 1) [27,43,44]. The first head bead carries a charge of $+e$, while the second head bead carries a charge of $-e$; the remaining two beads are uncharged [45]. Particularly, when modeling the negatively charged lipids, non-charged hydrophilic bead is used to take place of the first positive charged bead in lipid molecule [46]. The receptor molecule has the same conformation of lipid molecule [24,26,29], but its first two head beads (R) are uncharged and can interact with the ligand bead (L) *via* soft Lennard-Jones (LJ) potentials [25]. Besides, in order to model the lipid membrane of different cell types (e.g., cancer cell and macrophage cell), for the sake of simplicity, we just change the specificity of the receptors in the membrane, i.e., for receptors on cancer cell membranes, they can just interact with the ligand on the hydrophilic (or charged) nanoparticle surface, while for receptors on macrophage cell membranes, they can just interact with some hydrophilic and non-charged beads of the HSA.

The DPD is a coarse-grained simulation technique with hydrodynamic interaction [36]. The dynamics of the elementary units which are so-called DPD beads, is governed by Newton's equation of motion $\mathrm{d}\mathbf{v}_i/\mathrm{d}t = \mathrm{f}_i/m$. Typically, in the DPD, there are three types of pairwise forces acting on bead i by bead j: the conservative force, dissipative force, and random force. In the present work, the electrostatic force is introduced to take into account the electrostatic interactions between charged beads. The conservative force $\mathbf{F}_{ij}^C = a_{ij}(1 - r_{ij}/r_c)\hat{\mathbf{e}}_{ij}$ is used to model the repulsive interaction of beads i and j, where $r_{ij} = |\mathbf{r}_{ij}|$ is the distance between beads i and j, $\hat{\mathbf{e}}_{ij} = \mathbf{r}_{ij}/r_{ij}$ is the unit vector, r_c is the cutoff radius of the force, and a_{ij} represents the maximum repulsion interaction of beads i and j. For any two beads of the same type, we take the repulsive parameter $a_{ii} = 25$, and for any two beads of different types, we set the interaction parameter to denote the hydrophilic/hydrophobic property of the beads as follows [47–49]: $a_{ij} = 25$ if the two beads are both hydrophilic or both hydrophobic, while $a_{ij} = 100$ if one is hydrophilic and the other is hydrophobic (notice that the charged bead is hydrophilic). The dissipative force $\mathbf{F}_{ij}^D = -\gamma(1 - r_{ij}/r_c)(\hat{\mathbf{e}}_{ij}\cdot\mathbf{v}_{ij})\hat{\mathbf{e}}_{ij}$ and random force $\mathbf{F}_{ij}^R = \sqrt{2\gamma k_B T}(1 - r_{ij}/r_c)\zeta_{ij}\Delta t^{-1/2}\hat{\mathbf{e}}_{ij}$ are for thermostat, where $\mathbf{v}_{ij} = \mathbf{v}_i - \mathbf{v}_j$ is relative velocity between beads i and j, γ is the strength of friction, ζ_{ij} is a symmetric random variable with zero mean and unit variance, and Δt is the time step of simulation [36]. The electrostatic interactions were introduced into DPD simulations by Groot [37]. The soft potential in the DPD allows for the overlap between DPD beads, therefore, when charged DPD beads are modeled, this can lead to the formation of artificial ion pairs and cause the divergence of the electrostatic potential. To avoid this problem, Groot chose to spread out the charges using the distribution [37]: $\rho_e(r) = \frac{3}{\pi r_e^3}(1 - r/r_e)$ with $r < r_e$, where r_e is the electrostatic smearing radius, and is typically set as 1.6 r_c.