Biomaterials 52 (2015) 171–179

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Design strategy of cell-penetrating copolymers for high efficient drug delivery

Ye Li, Diwen Feng, Xianren Zhang*, Dapeng Cao*

State Key Laboratory of Organic-Inorganic Composites, Beijing University of Chemical Technology, Beijing 100029, China

ARTICLE INFO

Article history:
Received 15 September 2014
Received in revised form
18 December 2014
Accepted 20 January 2015
Available online

Keywords:
Cell-penetrating copolymer
Penetration mechanism
Dissipative particle dynamics simulation
Drug delivery carriers

ABSTRACT

Finding a highly effective drug delivery carrier with low cytotoxicity is essential for disease therapy. In this work, we design a cell-penetrating copolymer (CPC) carrier, in which the inspiration comes from cell-penetrating peptides that have both hydrophilic and hydrophobic residues and are capable of penetrating membranes without inducing membrane disruption. Further dissipative particle dynamics simulations indicate that the CPCs also have an effective penetration capacity. Importantly, we found that the penetration mechanism of the CPC is in a zipper way, i.e. the adjacent hydrophobic segments of the CPC could cross the membrane in a cooperative way. Moreover, we determine the optimal parameters for the CPC crossing lipid membrane, i.e. the hydrophobic segment length of the CPC is close to the membrane thickness, and the CPC has more segment number. Finally, by grafting the CPC with the optimal structure on the hydrophilic drug, we found that the CPCs can definitely help the hydrophilic drug penetrate the lipid membrane effectively, which is an excellent prototype of drug delivery carriers. It is expected that this work can provide the fundamental for further design of drug delivery carriers.

1. Introduction

At present, the cancer is one of the most devastating malignant diseases threatening human health [1]. However, multidrug resistance is a major obstacle for cancer therapy [2–4]. Traditional drug carriers have a number of limitations and disadvantages for drug delivery, such as off-target effect, poor water solubility, short circulation time, inconsistent stability, unfavorable biodistribution. Therefore, as the first step to overcome multidrug resistance, designing a highly effective drug delivery carrier becomes very urgent and important.

During the last decade, the potential of peptides for drug delivery into cells has been highlighted by the discovery of several cell-penetrating peptides. Cell-penetrating peptides are short sequences of amino acids (<30) and demonstrated to be efficient in entering the cells in a seemingly energy-independent manner. Their ability to cross biological membranes in a non-disruptive way without apparent toxicity is highly desired for increasing drug bioavailability. It has currently been verified in animal models that these peptides for the delivery of therapeutic agents with low toxicity. This property makes them extraordinarily good candidates as transporters for drug delivery [5–12]. This highlighted penetration capacity of cell-penetrating peptides is attributed to their special structures [13,14], most of which have both hydrophilic and hydrophobic residues.

Besides, except for the cell-penetrating peptides, the other amphiphilic materials also show the capacity for drug delivery. Among existing drug carriers, the spherical supramolecular nanoassemblies from amphiphilic block copolymers, called polymeric micelles, have attracted considerable attention in the drug delivery field, because their chemical composition, total molecular weight and block length ratios can be easily changed, which allows us to tailor the size and morphology of the micelles [15,16]. The structural characteristics of the amphiphilic block copolymers is that the hydrophilic blocks can resist protein adsorption and cellular adhesion, while the hydrophobic blocks effectively protect proteins against hydrolysis and enzymatic degradation. Moreover, the amphiphilic block copolymers exhibit good water-solubility, high drug loading capacity and low toxicity, which can prolonged circulation in the blood and enhanced accumulation in tumor tissue [17]. Besides, a number of studies have claimed that amphiphilic block copolymers can offer the most adequate and suitable means for designing functional drug delivery devices [18–22]. Gao et al.

* Corresponding authors.
E-mail addresses: zhangxr@mail.buct.edu.cn (X. Zhang), caodp@mail.buct.edu.cn (D. Cao).

http://dx.doi.org/10.1016/j.biomaterials.2015.01.046
0142-9612/

found experimentally that the amphiphilic block copolymers have cancer-targeting capability *via* $\alpha_V\beta_3$ integrins and controlled drug delivery [23]. Fang et al. also demonstrated that the pluronic block copolymers, which are amphiphilic synthetic polymers containing hydrophilic poly (ethylene oxide) blocks and hydrophobic poly (propylene oxide) blocks arranged in triblock structure, could be a potential vehicle for delivering hydrophobic chemotherapeutic drugs to MDR tumors [24]. In particular, recently reported intracellular environment-sensitive polymeric micelles can release the loaded adriamycin drug in pH-dependent way, which minimize the non-specific systemic spread of toxic drugs and maximize tumor-directed drug delivery efficiency [25–29]. Previous investigations also demonstrated that the hydrophilic block of the amphiphilic block copolymers modified with cationic charges can electrostatically interact with DNA or siRNA, and hydrophobic core can serve as a payload for hydrophobic drugs, making block polymers truly a promising multifunctional vehicle for both genetic and chemotherapy application [30].

In fact, numerous studies have demonstrated that the amphiphilic materials are extraordinarily effective candidates as transporters for drug delivery *via* controlling the drug release profile, inducing high antitumor activity with extremely low toxicity and avoiding clearance by the host defense system and uptake by normal tissue. However, the translocation mechanism of the amphiphilic materials for drug delivery is still poor and unclear. Moreover, the transport capacity of polymer–drug conjugates *in vivo* is still very low. Therefore, understanding the translocation mechanism of the amphiphilic materials for drug delivery can provide fundamental and reference for further designing excellent drug carriers [31–33]. To this end, in this work we combine the advantages of block copolymers and cell-penetrating peptides for drug delivery by designing the cell-penetrating copolymer (CPC), which are of similar structure as amphiphilic block copolymers featured with alternating hydrophilic and hydrophobic segments and also have the penetration capacity as the cell-penetrating peptides. We first use a dissipative particle dynamics (DPD) simulation [34–36] to investigate the translocation mechanism of the CPCs across the lipid membrane, and reveal the effective driving force and key factors for the penetration of CPCs crossing lipid membrane. By grafting the CPCs on the hydrophilic drug, we then design a CPC-based drug delivery prototype, which can effectively help the hydrophilic drugs penetrate the lipid membrane.

2. Computational details

2.1. Models

Here, CPCs are composed of alternating hydrophilic segments (P_H) and hydrophobic segments (P_T), and a typical structure is presented in Fig. 1. In order to explore the penetration mechanism,

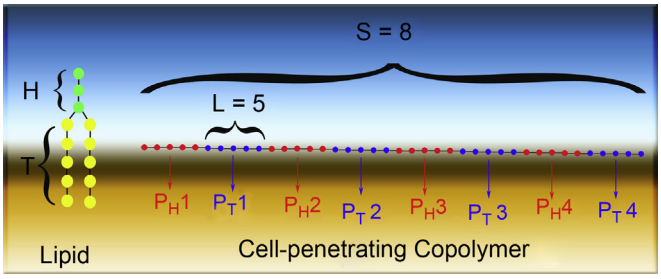

Fig. 1. Structure of the lipid and cell-penetrating copolymer (CPC). Lipid head is shown in green, lipid tail in yellow. Hydrophilic segments of the CPC are shown in red and hydrophobic segments in blue. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

we investigate the effects of hydrophilic-hydrophobic segment length (L), the number of the segments (S) and the CPC concentration on CPC penetration. A lipid molecule [37] is modeled by connecting a head group with three hydrophilic beads (H) to two hydrophobic tails of equal length, and each having five hydrophobic beads (T) (see Fig. 1). The coarse grained lipid model represents dimyristoyl-phosphatidylcholine (DMPC), and it can form stable bilayers and show typical phase behavior of the lipid bilayer. Water molecule is represented by a single bead (W).

2.2. DPD simulation

In this work, DPD simulations were performed to investigate the translocation pathway of CPCs into cellular membranes, aiming at finding the highly effective structures for CPCs as drug delivery. The DPD method has been extensively used to simulate the hydrodynamic behavior of complex fluids [34–36], in which the dynamics of DPD beads are governed by Newton's equation of motion, in a similar way like molecular dynamics (MD). The DPD has now become one of the most commonly used computer simulation techniques to study the biomembrane systems [38–48], especially on the interactions between the biomembranes and nanoparticles [39,44–47].

In the framework of DPD, beads i and j interact with each other *via* a pairwise additive force consisting of a conservative force F_{ij}^C, a dissipative force F_{ij}^D and a random force F_{ij}^R. Thus, the total force exerted on bead i can be expressed as $F_i = \sum_{i\neq j}(F_{ij}^C + F_{ij}^D + F_{ij}^R)$. The conservative force between beads i and j, which is soft and repulsive, is determined by $F_{ij}^C = a_{ij}\widehat{\mathbf{r}}_{ij}\max\{1 - r_{ij}/r_c, 0\}$ with a_{ij} the maximum repulsive force between particles i and j, $\mathbf{r}_{ij} = \mathbf{r}_j - \mathbf{r}_i$ ($\mathbf{r}_i$ and $\mathbf{r}_j$ are the positions of particles i and j), $r_{ij} = |\mathbf{r}_{ij}|$, $\widehat{\mathbf{r}}_{ij} = \mathbf{r}_{ij}/|\mathbf{r}_{ij}|$, and r_c the cutoff radius. For the first type of systems, the interaction parameters between the same species beads were set to $a_{TT} = a_{WW} = a_{HH} = 25$, and those between the different species were $a_{HW} = 25$, $a_{HT} = 50$, $a_{TW} = 80$. The interaction parameters greater than 25 correspond to a repulsive force, while those smaller than 25 correspond to attraction.

The dissipative force is determined by $F_{ij}^D = -\gamma(1 - r_{ij}/r_c)^2(\tilde{r}_{ij}\cdot v_{ij})\tilde{r}_{ij}$ with γ the friction coefficient, and $v_{ij} = v_i - v_j$ (v_i and v_j are their velocities). The random force also acts between each pair of particles by $F_{ij}^R = -\sigma(1 - r_{ij}/r_c)^2\theta_{ij}\tilde{r}_{ij}$, where σ is the noise amplitude and θ_{ij} is an uncorrelated random variable.

In the model of lipid molecules and the CPC, the interaction between neighboring beads within the same molecule is described by a harmonic spring force, given by $F_s = K_s(r_{ij} - r_{eq})\tilde{r}_{ij}$, where the spring constant K_S was set to $128k_BT$ and the equilibrium bond length r_{eq} was set to $0.7r_c$. The force constraining the variation of bond angle is given by $F_\varphi = -\nabla U_\varphi$ and $U_\varphi = K_\varphi(1 - \cos(\phi - \phi_0))$ with $\phi_0 = \pi$ and $K_\phi = 10.0$ for lipid molecules. For the CPC, we set $\phi_0 = \pi$ and $K_\phi = 10.0$ for hydrophilic segments of the CPC and $K_\phi = 100.0$ for the hydrophobic segments.

Without loss of generality, we selected the interaction cutoff radius r_c, the bead mass m, and the thermostat temperature k_BT to unity in our simulations. For all the systems, the periodic boundary conditions were implemented in three directions.

3. Results and discussion

3.1. The effective driving force for the penetration of the CPC

In order to explore the penetration mechanism of CPCs, we first study the effective driving force for a CPC crossing lipid membrane,