Journal of Colloid and Interface Science 522 (2018) 74–81

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

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

Regular Article

2,2′-Dithiodisuccinic acid-stabilized polyion complex micelles for pH and reduction dual-responsive drug delivery

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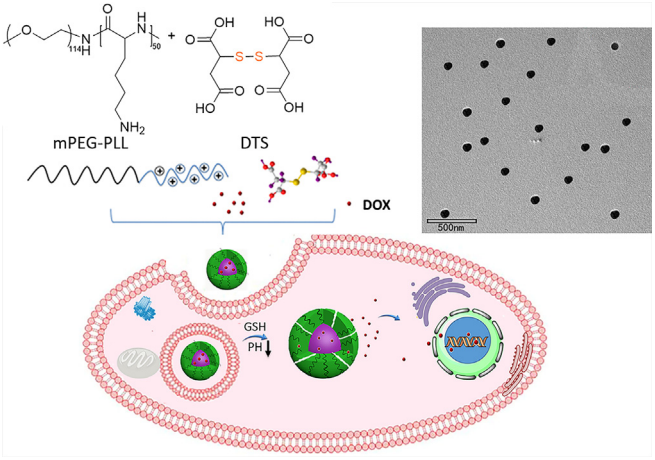
GRAPHICAL ABSTRACT

A new kind of PIC micelles consisting of a block copolymer mPEG-PLL and a disulfide-contained counterionic compound DTS was readily prepared for pH and reduction dual-responsive drug delivery.



ARTICLE INFO

Article history:
Received 7 January 2018
Revised 14 March 2018
Accepted 14 March 2018
Available online 15 March 2018

Keywords:
Polyion complex micelles
Drug delivery
pH responsiveness
Reduction responsiveness

ABSTRACT

In this work, a new kind of polyion complex (PIC) micelles with pH and reduction dual responsiveness was developed for effective intracellular drug delivery. The PIC micelles can be readily prepared by mixing a polycationic block polymer, methoxy poly(ethylene glycol)-*b*-poly(L-lysine) (mPEG-PLL), with a small molecule polyacid, 2, 2′-dithiodisuccinic acid (DTS) in aqueous media. The resultant PIC micelles are of uniform spherical shapes with hydrodynamic radii ranging from 65 to 75 nm based on different feeding ratios of mPEG-PLL and DTS. Interestingly, by using the small molecule polyacid DTS, the obtained PIC micelles show distinct pH-responsive disintegration in acid solution. Meanwhile, the PIC micelles were also assessed to be reduction-responsive due to the presence of disulfide bond in DTS. In view of these stimuli-responsive properties, the potential use of this PIC micelle as smart drug carrier was then investigated. Doxorubicin (DOX), a cationic anticancer drug, was loaded into the PIC micelles

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https://doi.org/10.1016/j.jcis.2018.03.049
0021-9797/

with high efficiency. *In vitro* drug release studies revealed that release of DOX from the PIC micelles was suppressed in neutral solutions due to the stable electrostatic interactions between PIC micelles and DOX, but could be accelerated in acidic solutions or under high GSH condition. Furthermore, flow cytometry and confocal laser scanning microscopy (CLSM) studies indicated that the DOX-loaded PIC micelles could be effectively internalized by MCF-7 human breast cancer cells and release the loaded DOX in intracellular environment. Consequently, the DOX-loaded PIC micelles were capable of inhibiting the proliferation of C26 murine colon cancer and MCF-7 human breast cancer cells in high efficiency, showing similar IC_{50} values as free DOX. Thus, this biocompatible PIC micelle may be promising for intracellular drug delivery.

1. Introduction

In the past two decades, tremendous efforts have been devoted to explore polymeric nanomedicines for anticancer therapy. It is well-known that, there are many potential benefits such as improved stability of drugs, prolonged blood circulation, and even acquired tumor targeting via "enhanced permeability and retention (EPR) effect", when using polymeric nanocarriers for anticancer drug delivery [1–4]. Nevertheless, significant challenges still remain in cell uptake and intracellular drug release of nanomedicines upon their arriving at the tumor site. To address this issue, polymeric nanocarriers that are stable in blood circulation but can release the payload drugs in response to intracellular biosignals (such as pH, reduced GSH and enzymes) have been widely investigated in recent years [5–9]. Among these nanocarriers, the polyion complex (PIC) micelles have received particular interests because of the advantages of simple preparation, efficient encapsulation of drugs and biosignal-sensitiveness [10,11].

The PIC micelles are typically formed by mixing an ionic block copolymer with a counterionic compound [10]. Initially, the PIC micelles were developed for encapsulation and delivery of ionic biopharmaceuticals such as DNA, RNA and proteins. The release of these ionic biopharmaceuticals from the PIC micelles is mainly governed by the ionic strength, pH values or ionic compounds in cellular compartments [10]. Usually, the PIC micelles are considered to be pH-sensitive due to the protonation-deprotonation of the opposite-charged ionic compounds. Thus, the pH-responsive structural changes of the PIC micelles are dependent on the pK_a of the ionic block copolymer in the PIC micelles [10,12,13]. To further improve the pH-sensitive release of ionic biopharmaceuticals, Kataoka et al. reported a new kind of PIC micelles based on a charge-conversional ionic block copolymer. The fast response of the polymer to a small pH change would facilitate the intracellular release of the loaded biopharmaceuticals [14–16]. In addition, Zhang and coworkers prepared a series of stimuli-responsive PIC micelles by incorporation of a stimuli-responsive small organic amphiphile into a counter-charged block copolymer [17,18]. The formed PIC micelles, also called supra-amphiphiles, can be sensitive to UV light, H_2O_2 or enzyme depending on the stimuli-responsive component in the organic amphiphiles.

Inspired by these works, herein, a small molecule polyacid, 2, 2′-dithiodisuccinic acid (DTS) was innovatively applied as the counterionic compound and mixed with a polycationic block polymer, methoxy poly(ethy1ene glycol)-*b*-poly(L-lysine) (mPEG- PLL) to form pH and reduction dual-responsive PIC micelles. The resultant PIC micelles were stable spherical nanoparticles with narrow polydispersity in water solution, while they showed stimuli-responsive disintegration of micellar structure in acidic solution or under reduction condition. Furthermore, the potential use of this kind of PIC micelles as smart drug carriers for intracellular drug delivery was demonstrated by using doxorubicin (DOX) as the model drug.

2. Experimental section

2.1. Materials and cells

Poly(ethylene glycol) monomethyl ether (mPEG. M_n = 5000) was purchased from Sigma-Aldrich China (Shanghai, China). Glutathione (GSH) and doxorubicin hydrochloride (DOX·HCl) were purchased from Aladdin Reagent (Shanghai) Co., Ltd. and Zhejiang Haizheng Pharmaceutical Co., Ltd., respectively. Tetrahydrofuran (THF) and *N,N*-dimethylformamide (DMF) were dried with calcium hydride (CaH_2) and subjected to distillation prior to use. All other chemicals and reagents were purchased from the Sinopharm Group Chemical Reagent Co., Ltd., and used without any pretreatment. The MCF-7 human breast cancer and C26 murine colon carcinoma cells were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). In all cell experiments, cancer cells were tested at 4–5 passages after thawing.

2.2. Instruments and methods

^{1}H NMR spectra were recorded on a Bruker AV300 NMR spectrometer. Dynamic light scattering (DLS) measurements were performed on a Wyatt QELS instrument with a vertically polarized He-Ne laser (DAWNEOS, Wyatt Technology) at 25 °C. The scattering angle was fixed at 90°. A single exponential fit was applied for the analysis of correlation function. The accuracy is better than 2% (the mean size of NIST latex standard). The solution was filtered with a 0.45 μm filter before measurement. Transmission electron microscopy (TEM) measurements were performed on a JEOL JEM-1011 transmission electron microscope with an accelerating voltage of 100 kV. A drop of the nanoparticle dispersion (0.1 mg mL^{-1}) was deposited onto a 200-mesh copper grid coated with carbon and allowed to dry in air at 25 °C before measurements. The molecular weights and polydispersities ($Đ$) of mPEG and mPEG-PZLL were characterized by gel permeation chromatography (GPC), which were carried out on a Waters 505 GPC instrument equipped with three Waters Styragel columns (HT3, HT4, and HT5) and a differential refractometer detector. The DMF (containing 0.01 M LiBr) was used as the eluent with a flow rate of 1 mL min^{-1} and the molecular weights were calibrated with a series of polystyrene standards.

2.3. Synthesis of mPEG-PLL

The mPEG-PLL block copolymer was synthesized using a method described in the previously reported literature [19]. Briefly, 9.0 g of ε-benzyloxycarbonyl-L-lysine *N*-carboxyanhydride (ZLL-NCA, 29.41 mmol) and 2.94 g of mPEG-NH_2 (containing 0.59 mmol amino groups) were dissolved in 100 mL of dried DMF in a flame-dry flask. The polymerization was performed at 25 °C for 3 days. Then, the solution was precipitated into 1000 mL of diethyl ether for three times. The obtained solid was dried under vacuum at room temperature (yield: 72.3%).