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Preparations of hyperbranched polymer nano micelles and the pH/redox controlled drug release behaviors

Zhang Liping, Zhou Yamin, Shi Gang, Sang Xinxin, Ni Caihua *

Key Laboratory of Synthetic and Biological Colloids, Ministry of Education, School of Chemical and Material Engineering, Jiangnan University, Wuxi 214122, China

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ABSTRACT

Hyperbranched polymer nano micelles (NMs) were prepared through a nucleophilic ring opening polymerization between cystamine and polyethylene glycol diglycidyl ether, followed by a reaction of amino groups and dimethyl maleic anhydride. The NMs showed spheric morphologies with hydrodynamic diameters of 106–120 nm. Doxorubicin was loaded in the NMs with loading rate as high as 15.38 wt%; The NMs possessed negative zeta potentials in aqueous solutions of pH 7.4 due to the carboxyl ions on the particle surfaces, but the zeta potentials were converted to positive ones due to the hydrolysis of amide bonds at pH 5.0–6.5, leading to the leaving of carboxyl groups and remaining of amino groups. The disulfide bonds in cystamine were designed in the hyperbranched polymer structures of the NMs, and bonds could be broken by a reducing agent L-glutathione (GSH) (10 mM), resulting in a targeted drug release. The smart NMs displayed good biodegradability and biocompatibility, and they could be potentially used in drug controlled release field.

1. Introduction

Polymeric nano micelles (NMs) have promising applications as drug delivery systems in cancer therapy since they have ultrasmall volumes, can pass through tissue interstice, and can be absorbed by particular cells without being removed by phagocytes [1–22].

Tumor cells have special pH and reduction environment. The concentration of glutathione (GSH) in the cell cytoplasm (1 mM–10 mM) is 1000 times higher than that in blood plasma (2 μM) [23–25]. The pH in blood was 7.35–7.45, and in the tumor tissue gap and endosomes the pHs were 6.5–6.8 and 4.5–5.0 respectively [26].

Desirable nano micelles need to be stable in the blood, with excellent anti-protein non-specific adsorption. Once enter into the gap of tumor tissue cells, the micelles can pass through the cell membranes, and release drugs by the stimulus of environmental pH and reducing agents. Therefore, preparations and applications of pH and reduction sensitive nano micelles have attracted growing attentions [27–30].

Hyperbranched polymers have particular structures and unique properties such as high solubility, low viscosity and three-dimensional spherical structure [31–33]. The large number of cavity structures are capable of accommodating more drugs than those of linear polymer nano micelles.

There were some reports on hyperbranched polymers which applied in drug delivery fields. Wang et al. prepared a temperature-responsive PEG-based branched polymer with disulfide bonds in its backbone via reversible addition–fragmentation chain transfer (RAFT) [34]. Hu et al. fabricated self-reporting theranostic drug nanocarriers based on hyperbranched polyprodrug amphiphiles [35]. Liu et al. synthesized a new amphiphilic hyperbranched homopolymer and realized its aqueous self-assembly to form spherical micelles which could be exploited to rapidly deliver hydrophobic drugs into the nuclei of tumor cells [36]. However, these hyperbranched polymers contained non-biodegradable polymer chains, was non pH sensitive and involved complicated synthetic processes.

In this study we designed and prepared pH/redox sensitive hyperbranched polymer nano micelles (NMs) through a nucleophilic ring opening polymerization, followed by the reaction of amino groups and dimethyl maleic anhydride. It is expected that the hyperbranched structural can improve drug loading rate so as to enhance efficacy of the NMs. The amide bonds are stable at pH 7.4 and will be broken at pH 6.5–5.0, so that the NMs can enter into tumor cells easily. The disulfide bonds are involved in the hyperbranched polymer structures of the NMs, and they are stable in physiological conditions, but will be destroyed by the reducing agent GSH with the high concentration in tumor cells, leading to targeted drug release. The preparations, characterizations and controlled release of the NMs were studied.

2. Experimental

2.1. Materials

Cystamine dihydrochloride (Cys•HCl), L-glutathione (GSH) and Bovine serum albumin (BSA) were bought from Sigma-Aldrich;

* Corresponding author.
E-mail address: nicaihua2000@163.com (C. Ni).

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Polyethylene glycol diglycidyl ether (PEGDE) was purchased from Guangzhou Aobao Chemical Technology Co. Ltd.; Doxorubicin hydrochloride (DOX•HCl) was purchased from Shanghai Hao Cloud Chemical Technology Co. Ltd.; 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), RPMI-1640 medium and fetal bovine serum (FBS) were purchased from TRIVD Biotech INC. All the other reagents were purchased from Sinoppharm Chemical Reagent Co. Ltd., unless otherwise stated.

2.2. The preparation of pure cystamine

Cystamine dihydrochloride (11.25 g, 0.05 mol) was dissolved in the mixed solvents of 16 mL of deionized water, 60 mL of ether and 24 mL of tetrahydrofuran under stirring. 66.7 mL of NaOH solution (40w%) was added dropwise to the above mixture under magnetic stirring for 1 h. The upper organic phase was separated from water phase, and was dried with 4 g of NaOH. The mixture was vortexed in a vacuum to remove volatile ether and tetrahydrofuran, giving 6.2 g of colorless cystamine with a yield of 75.5%.

2.3. Preparation of hyperbranched polymer nano micelles (NMs)

Cystamine (0.616 g) was dissolved in 8 mL of ultrapure water, a mixture of PEGDE (2.873 g) and ultrapure water (8 mL) was added under magnetic stirring at 60 °C for 24 h. The product solution was poured into a dialysis bag (MWCO 3500), and was dialyzed against ultrapure water for 3 days (the dialysate was changed every 4 h). A hyperbranched polymer was obtained and designated as CP.

The hyperbranched polymer (CP, 0.5 g) was dissolved in 10 mL deionized water, and the pH of the sample solution was adjusted to 8.5 with 0.1 M NaOH solution. A solution of 0.3 g dimethyl maleic anhydride (DMMA) in 5 mL of *N*,*N*-Dimethylformamide (DMF) was added dropwise into the hyperbranched polymer solution at temperature of 25 °C under stirring for 48 h. After the reaction, the solution was transferred to a dialysis bag (MWCO = 3500 Da) and was dialyzed against 0.1 M NaOH solution (pH 8–9) for 72 h. The solution gradually showed blue milk light during the dialysis process, indicating the formation of micelles. Afterwards, the sample was freeze-dried to get a product named as CPD. The similar preparations were carried out using maleic anhydride (MA) and succinic anhydride (SA) replacing DMMA respectively, yielding products of CPM and CPS respectively.

2.4. Characterizations

The structures of the hyperbranched polymers were characterized by Fourier transform infrared spectra (FTIR, Nicolet 6700, USA). Nuclear magnetic resonance (^{1}H NMR, AVANCE III HD 400 MHz) was employed to characterize the polymer structures. The molecular weight and the polydispersity (PDI) of the polymers were measured by gel permeation chromatography (GPC, HLC-8320GPC EcoSEC, Japan). Hydrodynamic diameters (D_h) and the distribution of the NMs were measured by Laser Light Scattering (DLS, ALV/DLS/SLS-5022F, Germany); Zeta potentials were determined by a zeta potential instrument at 25 °C (Zeta PALS Malvern Instruments, UK). Reduction responsiveness was verified by measuring NMs particle sizes and fluorescence intensity changes of pyrene used as the probe in the micelle solution. The morphologies of NMs were observed by scanning electron microscopy (SEM, S-4800, Japan).

2.5. pH sensitivity test

The polymeric micelles CPD, CPM, CPS were incubated at 37 °C for 3 h in phosphate buffer solutions of pH 7.4, 6.5 and acetate buffer solution of pH 5.0 respectively, then zeta potentials were recorded on a zeta potential instrument.

2.6. Reduction sensitivity test

Reduction sensitive behavior was investigated with two methods. The CPD micelles were incubated with GSH aqueous solution (10 mM), and the size change was recorded at different incubating time.

The CPD micellar solution 10 mL (0.1 mg/mL) was added to 5 mL of pyrene acetone solution (0.5 mg/L). The solution was shaken evenly at the room temperature and allowed to stand for 24 h for volatilizing the acetone. Then, the GSH solution was added and the mixture was incubated at 37 °C under shaking. The fluorescence intensities were measured with a spectrophotometer at different time points, the emission wavenumber was fixed at 395 nm and the slit width was set at 5 cm.

2.7. Evaluation of ability to accept protons

The NMs sample was dissolved in 0.1 M NaCl solution to give a concentration of 2 mg/mL. The pH of the sample solution was adjusted to 11.0 with 1 M NaOH solution, and was titrated with 0.1 M HCl solution and the titration curve was plotted. Here 0.1 M NaCl solution was used as the control group.

2.8. Drug loading

DOX solution (10 mL, 0.1 mg/mL) was added dropwise to an aqueous solution of CPD (20 mL, 0.5 mg/mL) under stirring for 24 h at the room temperature, and was transferred into a dialysis bag (MWCO 3500) for dialysis 24 h, the drug-loaded micelles were filtrated with 0.45 μm microporous membrane, and finally were freeze-dried. The drug loading rate (LR) and encapsulation efficiency (EE) of the drug were calculated as follows:

$$LR(\%) = W_D/W_S \times 100\% \quad (1)$$

$$EE(\%) = W_D/W_{TD} \times 100\% \quad (2)$$

where W_D, W_S and W_{TD} referred to the weight (g) of the drug in the NMs, the NMs, and the total drug added respectively.

2.9. In vitro drug release study

A dialysis bag (MWCO 3500) containing CPD-DOX solution (5 mL, 1 mg/mL) was placed in 250 mL of PBS solution with particular pH and different concentrations of GSH under continuous shaking

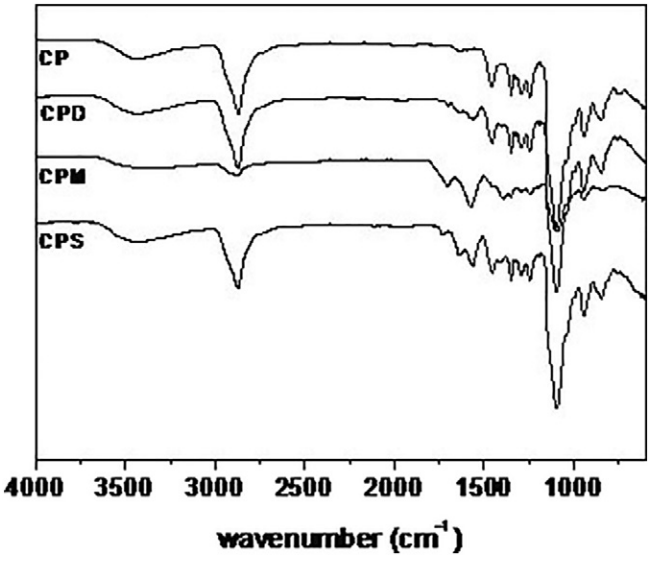

Fig. 1. FTIR spectra of the hyperbranched polymers of CP, CPD, CPM and CPS.