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ScienceDirect
Journal of Controlled Release 126 (2008) 130–138
journal of controlled release
www.elsevier.com/locate/jconrel

Physicochemical characteristics of pH-sensitive poly(L-Histidine)-*b*-poly (ethylene glycol)/poly(L-Lactide)-*b*-poly(ethylene glycol) mixed micelles

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Received 15 October 2007; accepted 26 November 2007
Available online 4 December 2007

Abstract

A novel pH-sensitive polymeric micellar system composed of poly(L-Histidine)-*b*-poly(ethylene glycol) and poly(L-Lactide)-*b*-poly(ethylene glycol) block copolymers was studied by dynamic/static light scattering, spectrofluorimetry and differential scanning calorimetry. The mixed micelles displayed ultra pH sensitivity which could be tuned by varying the mixing ratio of the two polymers. In particular, mixed micelles composed of 25 wt.% poly(L-Lactide)-*b*-poly(ethylene glycol) exhibited desirable pH dependency which could be used as a drug delivery system that selectively targeted the extracellular pH of acidic solid tumors. Micelles were quite stable from pH 7.4 to 7.0 but underwent a two-stage destabilization as pH decreased further. A significant increase in size and aggregation number was observed when pH dropped to 6.8. Further disruption of the micelle core eventually caused phase separation in the micelle core and dissociation of ionized poly(L-Histidine)-*b*-poly(ethylene glycol) molecules from the micelles as pH decreased to 6.0. Increased electrostatic repulsions which arise from the progressive protonation of imidazole rings overwhelming the hydrophobic interactions among uncharged neutral blocks is considered to be the mechanism for destabilization of the micelle core.

Keywords: Polymeric mixed micelles; pH sensitive; Drug delivery; poly(L-Histidine); Tumor pH

1. Introduction

Amphiphilic block copolymers consisting of a hydrophilic segment and a hydrophobic segment self-assemble into polymeric micelles having a hydrophobic core structure stabilized by a hydrophilic shell in aqueous solution. In recent years, polymeric micelles have been extensively investigated for pharmaceutical applications because of their attractive features as drug delivery vehicles [1–6]. Polymeric micelles mimic aspects of the biological transport system in terms of structure and function. A hydrophilic surface prolongs their blood circulation while smaller size (typically 20–200 nm in diameter) prevents recognition and uptake by the reticuloendothelial system. As a result, these nanovehicles can have relatively long circulation times after intravenous administration and can passively accumulate in solid tumors for example due to the enhanced permeability and retention effect [7]. Furthermore, the critical micelle concentration of polymeric micelles is usually much lower than low molecular weight surfactant micelles, which ensures improved physical stability against dilution after injection into the blood stream.

An important issue in determining the effectiveness of a micellar drug carrier is its efficient drug release after reaching target sites (*i.e.*, cancers). This challenge has motivated the development of micelle systems with a triggered release mechanism which enables the carriers to release drug in response to specific external or internal stimuli such as temperature [8,9], pH [10,11], ultrasound [12,13] or enzymes[14]. Among these stimuli, changes in acidity are particularly useful in the development of micellar drug carriers for treating solid-tumor cancers. First, the relatively acidic tumor extracellular pH (pH_e) is a distinguishing phenotype of solid tumors from surrounding normal tissues. The measured pH_e values of most solid tumors in patients range from pH 5.7 to pH 7.2 [15] while normal blood remains well-buffered and

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doi:10.1016/j.jconrel.2007.11.014

constant at pH 7.4. Moreover, changes in pH are also encountered once the micelle enters cells via endocytosis pathways where pH can drop as low as 5.5–6.0 in endosomes and approaches 4.5–5.0 in lysosomes. In order to take advantage of the acidic nature of tumor tissue and endocytic vesicles, two strategies have been used thus far to introduce pH sensitivity into a micellar system. One approach is to incorporate an acid labile linkage between the drug and the polymer forming the micelles. The cleavage of such chemical bonds by acidic pH can accelerate antitumor drug release from nano-vehicles [10]. Another approach is to incorporate pH-sensitive groups such as amines or carboxylic acids into the block copolymers so that the carriers undergo structural destabilization at acidic pH by protonation of these groups [11,16].

Copolymers with hydrophilic blocks such as PEG and hydrophobic blocks composed of biodegradable poly(amino acids) have the strong potential to be used as drug carriers due to their non-toxicity and biocompatibility. Recently, our group developed such a novel pH-sensitive poly(amino acid) based diblock copolymer—poly(L-Histidine) ($M_n \sim 5000$)-*b*-poly (ethylene glycol) (M_n 2000) (referred as PH-PEG) [17]. Poly (L-Histidine) ($M_n \sim 5000$) was synthesized by ring opening polymerization of L-Histidine *N*-carboxyanhydride and coupled to poly(ethylene glycol) (M_n 2000) via an amide linkage. The polymer exhibited pK_b around 7.0 and a buffering pH region of pH 5.5–8.0 due to the amphoteric nature of imidazole rings on the PH blocks. Polymer micelles constructed from PH-PEG copolymer were about 110 nm in size at pH 8.0 but began to dissociate below pH 7.4. In order to tailor the triggering pH of the polymeric micelles to the more acidic extracellular pH of tumors while improving their stability at pH 7.4, another biocompatible polymer, poly(L-Lactic acid) (M_n 3000)-*b*-poly (ethylene glycol) (M_n 2000) (referred as PLLA-PEG) was blended with PH-PEG to form mixed micelles [18]. The anticancer drug doxorubicin (DOX) was successfully incorporated into the mixed micelles with a relatively high loading content (15–17 wt.%) and the mixed micelles containing 25 wt.% PLLA-PEG was found to be selectively responsive to extracellular tumor pH. However the physicochemical nature of the mixed micelles and the mechanism of pH dependent structural transitions still remained unexplored. In this work, the pH sensitivity and interior structural features of the mixed micelles were systematically examined, including morphology and anisotropy, thermodynamic and kinetic stability, miscibility of PH and PLLA blocks in the micellar core and pH dependent structural transitions. Based on experimental results, the destabilization mechanism of the mixed micelles is discussed in detail below.

2. Experimental section

2.1. Materials

Z-His(Bzl)-OH, isopropylamine, triethylamine, PEG (M_n: 2000 Da), diethyl-aminoethyl, (DEAE) Sephadex A-25, potassium tetraborate, ammonium bicarbonate, *N*-hydroxysuccinimide (NHS), *N*,*N*'-dicyclohexylcarbodiimide (DCC), anhydrous dimethylformamide (DMF), anhydrous 1,4-dioxane and dimethylsulfoxide (DMSO) were purchased from Sigma Co. Thionyl chloride was purchased from Fluka Co. Potassium *tert*-butoxide and ethyl bromoacetate were purchased from Acros Organics. Pyrene and diphenyl hexatriene (DPH) were purchased from Sigma Co. and used as received.

2.2. Polymer synthesis

(1) *poly(*L-Histidine*)-b-poly(ethylene glycol)*. Synthesis and purification of poly(L-Histidine) (M_n: ~5000 Da)-*b*-poly (ethylene glycol) (M_n: 2000 Da) followed the methodology established by our group, which can be found in details elsewhere [17,19]. The molecule weight of the poly(L-Histidine) block determined from ^{1}H NMR was 5200 Da (see Fig. S1 in the Supporting information).

(2) *poly(L-Lactic acid)-b-poly(ethylene glycol).* PLLA-PEG diblock copolymer was synthesized by ring opening polymerization of L-Lactide initiated by hydroxy group of PEG monoacid (M_n: 2000 Da) in the presence of stannous octoate as a catalyst [20]. The molecule weight of the poly(L-Lactide) block as determined from ^{1}H NMR was 2860 Da (Fig. S2).

2.3. Preparation of polymeric micelles

Since the polymer mixtures are not readily dissolved in water, a dialysis method was employed to fabricate polymeric micelles [2]. 20 mg of PH-PEG and PLLA-PEG mixtures were weighed respectively at predetermined mixing ratios and dissolved in 3 mL DMSO. Subsequently, 2 mL phosphate buffer (pH 8.0, 10 mM) was added dropwise into the solution. The resulting solution was vigorously stirred for half an hour and then transferred into a pre-swollen dialysis membrane (SPECTRA/POR; MWCO 3500) and dialyzed against 10 mM phosphate buffer (pH 9.0). The outer phase was replaced with fresh buffer solution at 1, 2, 4, 6, and 12 h. After 24 h, the micelle solution inside the membrane was recovered. The yield of mixed micelles from dialysis was c.a. 90 w/w%. Afterwards, the micelle solution was diluted and adjusted to a predetermined pH with a CORNING 443 i pH meter by adding 1 N HCl stock solution.

2.4. Dynamic light scattering

Dynamic light scattering (DLS) measurements were carried out with a Brookhaven Instruments Corp. system consisting of a BI-200SM goniometer and a BI-9000AT autocorrelator. The solutions were filtered prior to measurements using a 0.80-μm disposable membrane filter. The results were analyzed by the constrained regularized CONTIN method to yield information on the distribution of the characteristic line width (Γ). The normalized distribution function of the characteristic line width $<\Gamma>$ so obtained can be used to determine an average apparent diffusion coefficient.

$$D_{app} = \Gamma / q^2 \quad (1)$$

where $q = 4\pi n \sin(\theta/2)/\lambda$ is the magnitude of the scattering wave vector.