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Non-enzymatic and enzymatic degradation of poly(ethylene glycol)-*b*-poly-(ε-caprolactone) diblock copolymer micelles in aqueous solution

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ABSTRACT

The non-enzymatic and enzymatic degradation behaviors of the monomethoxy-poly(ethylene glycol)-*b*-poly(ε-caprolactone) diblock copolymers (MPEG–PCL) micelles in aqueous solution were investigated by DLS, ^{1}H NMR, SEC and HPLC. It is found that the degradation mechanism of MPEG–PCL micelles in aqueous solution in non-enzymatic case is quite different from that in the presence of enzyme. In non-enzymatic case, the degradation induced by acidic catalysis was not found in low pH aqueous solution but the degradation of the micelles occurred under neutral and basic conditions. The degradation of MPEG–PCL micelles first happens near the interface region of the MPEG shell and PCL core, leading to the part detachment of PEG chains. With increasing degradation time, the degradation inside the PCL core with a random scission on PCL chains occurred. Compared with non-enzymatic degradation, the enzymatic degradation of MPEG–PCL micelles is much fast and the degradation rate of MPEG–PCL micelles is proportional to either the micelles or the enzyme concentration in a certain range. Based on the micelle degradation behaviors that we observed, a possible mechanism for the enzymatic degradation of the MPEG-*b*-PCL micelles including PCL core erosion which results in cavitization of micellar core and micellar dissociation is proposed.

1. Introduction

Polymer micelles, made of biocompatible and biodegradable amphiphilic block copolymers, such as poly(lactide)–poly(ethylene glycol) (PLA–PEG) [1], poly(caprolactone)–poly(ethylene glycol) (PCL–PEG) [2–4] and poly(caprolactone-*co*-lactide)–poly(ethylene glycol) (PCLLA–PEG) [5,6], have been the subject of scientific attention in recent years. These micelles with a core-shell structure are normally prepared by a self-assembly strategy using amphiphilic block copolymers in a selective solvent [7–11], and such core-shell structure micelles can incorporate lipophilic drugs into their cores and release the drug in a controlled manner at a later stage, making them a potential carrier for poor water solubility drugs [12].

Although these micellar systems have been thoroughly studied on the preparation method, size control, modification, drug loading and drug release behavior [13], the understanding on the degradation behaviors of polymeric micelles is still limited. Moreover, the divergence in their degradation mechanism is obviously present. For example, Li et al. reported the enzymatic degradation of the films prepared by PCL-containing block copolymers [14–19] and they found that the content of PEG in the copolymer would not affect the degradability of the PCL segment [15,16]. However, they have not referred to the degradation of the copolymer micelles yet. Belbella et al. studied the degradation of D,L-PLA nanospheres in different pH solutions and they pointed out that the hydrolysis was much more catalyzed in acidic and basic media with respective "random scission" and chain-end cleavage" mechanisms than in neutral medium [20]. The degradation process of giant and flexible worm micelles prepared from PEO–PCL has also been studied and it was found that the worm micelles could be spontaneously shorten to spherical micelles by chain-end hydrolysis of the PCL [21]. Wu et al. developed a novel method to study the enzymatic biodegradation of PEO–PCL nanoparticles and micelles in the presence of the lipase PS (from *Psedomonas cepacia*) by laser light scattering (LLS) [22–24]. They found that the degradation was a first-order reaction [22–25] and the initial biodegradation rate was independent of the micelles concentration for a given enzyme concentration [22–24]. They also pointed out that the lipase PS "ate" the PCL micelles in a one-by-one manner [22–25], though the conclusion, they

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Table 1
The properties of the MPEG–PCL block copolymers and their micelles.

Sample	NP2K4K	NP4K20K	NP10K30K
CL/EG[a]	0.77	1.93	1.16
CL/EG[b]	0.76	1.90	1.18
M_n[c]	4.7k	18.6k	32k
M_w[c]	8.1k	30.5k	49.4k
M_w/M_n[c]	1.72	1.64	1.54
Micellar size/nm[d]	35.4 ± 2.5	72.4 ± 2.3	92.3 ± 2.1
Micellar size/nm[e]	49.2 ± 2.8	89.4 ± 3.2	139.0 ± 4.9
P.D.[f]	0.17	0.21	0.12

[a] Molar ratio in feed.
[b] Determined by ^{1}H NMR.
[c] Determined by SEC.
[d] prepared in ratio of polymer/acetone/water 40/2/40 (mg/mL/mL).
[e] prepared in ratio of polymer/acetone/water 100/2/25 (mg/mL/mL).
[f] P.D.: polydispersity of the micelles.

thought, "should be reconsidered" after introducing a new monitor method of pH in situ examination [24]. Lately, Myrra et al. investigated the chemical and enzymatic degradations of short monodisperse oligo(ε-caprolactone) (OCL) and its amphiphilic block oligomer with short chain PCL and PEG, and they concluded that the PEG-*b*-OCL micelles are stable systems in buffer, and the degradation of OCL was markedly accelerated by the presence of lipase [26]. In our previous work, the non-enzymatic degradation behavior of PEG–PCL triblock copolymer micelles in aqueous solution at room temperature was studied [27].

In this paper, we used the MPEG–PCL diblock copolymer micelles as a model to study their non-enzymatic and enzymatic degradation behaviors in aqueous solution at 37 °C by dynamic light scattering (DLS), proton nuclear magnetic resonance (^{1}H NMR), size exclusion chromatography (SEC), high performance liquid chromatography (HPLC) and transmission electron microscopy (TEM) techniques in order to gain further insight into the degradation of PEG–PCL micelles.

2. Experimental section

2.1. Materials

ε-Caprolactone (ε-CL) (Aldrich, USA) was purified by drying over CaH_2 and distilled under reduced pressure. Monomethoxy-poly(ethylene glycol) (MPEG), with molecular weights of 2000, 4000, and 10 000 g/mol were obtained from Jinling Petroleum Co., Jiangsu, China, and vacuum-dried at 50 °C for 24 h before use. Stannous octoate (Sigma) and 6-hydroxycaproic acid (Alfa Aesar) were used as-received. Lipase AY with the activity of about 2 U/mg (lipase from *Candida cylindracea*, Fluka) was purified by freeze-drying. All other chemicals were of analytical grade and used without further purification.

2.2. Synthesis of MPEG–PCL diblock copolymers

MPEG–PCL diblock copolymers were synthesized by a ring-opening copolymerization as previously described [5,27]. Briefly, a predetermined amount of CL was added into a polymerization tube containing MPEG and a small amount of stannous octoate (0.1% wt/wt). The tube was then connected to a vacuum system, sealed off, and placed into an oil bath at 130 °C for 48 h. After the polymerization was complete, the crude copolymers were dissolved with chloroform and precipitated into an excess amount of diethyl ether to remove the unreacted monomer and oligomer. The precipitates were then filtered and washed with diethyl ether several times before thoroughly dried at reduced pressure.

2.3. Preparation of MPEG–PCL micelles

MPEG–PCL diblock copolymer micelles were prepared by a precipitation method. Copolymer 100 mg was dissolved in 5 mL of acetone, and the solution was added dropwise into 25 mL of distilled water under moderate stirring at 25 °C to produce an aqueous suspension. The acetone in suspension was then removed under reduced pressure or by dialysis in water. The suspension was filtered with a microfilter of pore size 650 nm to remove the polymer aggregates and the larger micelle aggregates.

2.4. Degradation of MPEG–PCL micelles

The degradation behaviors were studied by two different ways: with and without the presence of lipase AY. In the non-enzymatic experiment, in each 25 mL bottle, 10 mL suspensions of MPEG–PCL micelles (about 4 mg/mL) was added, and the volume was adjusted to 20 mL with buffer solution (KH_2PO_4, 0.1 N pH 4.4). The medium pH can also be adjusted to the required value (4.44, 7.40 and 10.20) by addition of 4 N NaOH. These bottles were then stored at 37 °C in dark. At determined intervals, samples were taken out from the bottles for analyses. In the enzymatic biodegradation experiment, a proper amount of lipase AY was added into the polymeric micelle dispersion to start biodegradation. The biodegradation was conducted at 37 °C both inside the DLS cuvette to in situ measure the effective diameter and light scattering intensity with time and in a big container to collect the samples for other tests simultaneously.

2.5. Characterization of copolymers and the micelle degradation products

SEC measurements were performed at room temperature on a Waters 515 systems equipped with a Wyatt Technology Optilab

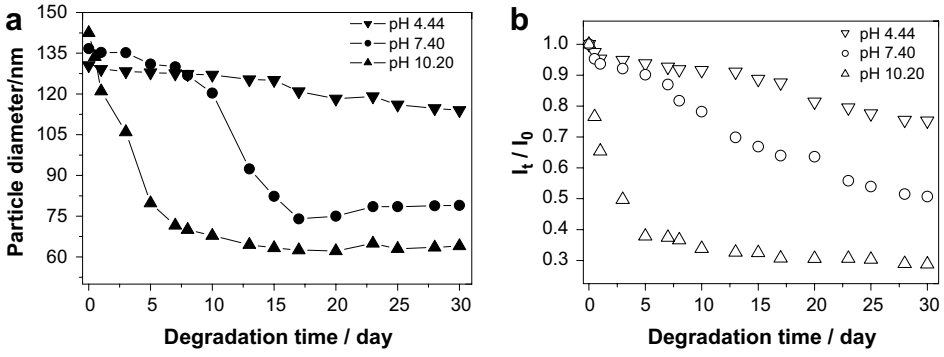

Fig. 1. (a) Micellar size and (b) light scattering intensity changes of NP10K30K micelles in different pH media at 37 °C during the non-enzymatic degradation process.