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ORIGINAL PAPER

Effect of block lengths on the association behavior of poly(L-lactic acid)/poly(ethylene glycol) (PLA–PEG–PLA) micelles in aqueous solution

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Abstract A series of poly(L-lactic acid)/poly(ethylene glycol) triblock copolymers with a PLA–PEG–PLA architecture were synthesized by a ring-opening polymerization (ROP) process. The copolymers were characterized by ^{1}H NMR and GPC. The total number average molecular weights were in the range of 4,700–50,000, whereas the degrees of polymerization of the PLA and PEG blocks varied from 15 to 359 and from 68 to 136, respectively. The self-association of these copolymers in aqueous environment was studied by emission fluorescence spectroscopy of anilinonaphthalene probe and the critical association concentration (CAC) of the copolymers was measured. It was found that the micellization process of these copolymers was mainly determined by the length of the hydrophobic LA block, while the length of the hydrophilic PEG block had little effect. Furthermore, the low CAC values of the copolymers suggest that the copolymers form stable supramolecular structures in aqueous solutions.

Keywords Poly(lactic acid) · Triblock copolymer · Block length · Polymeric micelles

Introduction

Biodegradable diblock copolymers of poly(ethylene glycol) (PEG) and poly(lactic acid) (PLA–PEG), and their corresponding triblocks are widely used for fabricating nanoparticles (NPs) as drug delivery carriers. The amphiphilic character of these copolymers enables the formation of micelles in water through a self-assembly process: the hydrophobic PLA blocks aggregate to form a core reservoir for hydrophobic drugs and the hydrophilic PEG segments serve as the shell projecting into the aqueous environment [1–3]. In recent years, most research has focused on the drug encapsulation and release behavior [4]. In contrast, only a few studies have been performed on the micellization properties of PLA/PEG block copolymers [2, 5]. It is known that the micelle assembly is controlled by the relative molecular weight of the hydrophobic and hydrophilic blocks. However, not much is known about how the ratio of hydrophobic and hydrophilic blocks influences the micellization behavior of copolymers. In this study, a series of PLA–PEG–PLA triblock copolymers with various block lengths and molecular weights were synthesized. The characterization of the copolymers, the formation of micelles and their critical association concentration (CAC) in aqueous solutions are reported. The objective of this study is to elucidate the effect of block lengths on the CAC values of the micelles, which is of great interest for applications in the field of drug delivery.

Experimental

Polymer synthesis and characterization

PEG was dried at 40 °C in vacuum overnight prior to use. Toluene was dried by storing with metal sodium in a bottle.

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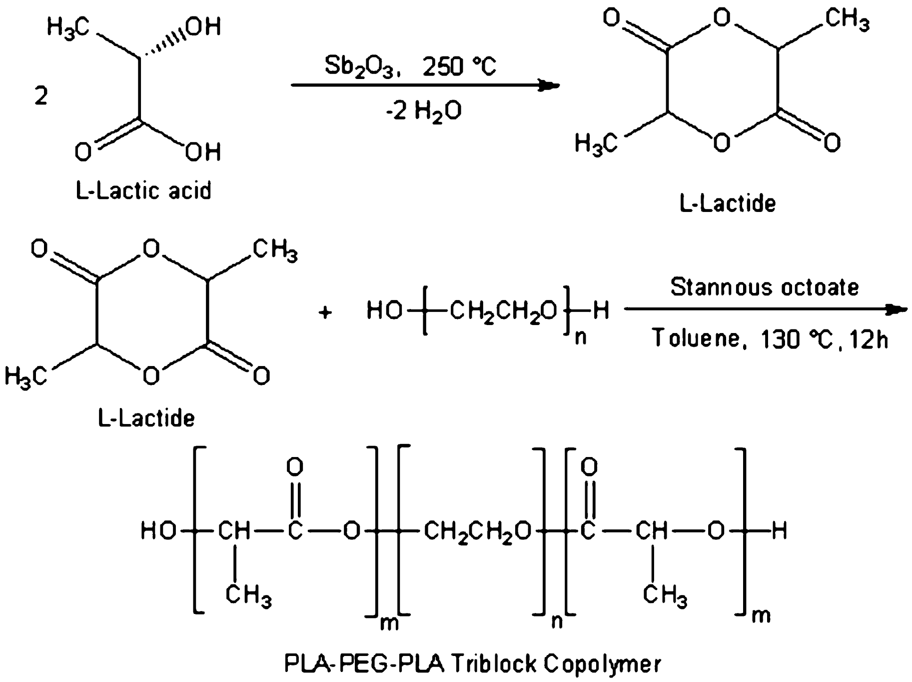
Scheme 1 Synthesis of L-lactide and PLA–PEG–PLA triblock copolymers



Synthesis of L-lactide

L-Lactide was synthesized from L-lactic acid, using antimony trioxide (Sb_2O_3) as a catalyst. The reaction was composed of two steps: (a) oligomerization at 180–200 °C, and (b) depolymerization and then dimerization at 250 °C. The produced L-lactide was recrystallized three times from ethyl acetate and dried in vacuum at 40 °C overnight before use (Scheme 1).

Synthesis of PLA–PEG–PLA triblock copolymers

Triblock copolymers were synthesized by a ring-opening polymerization (ROP) process [3]. In brief, a given amount of purified L-lactide was charged into a 100 mL round bottle flask containing pre-dried PEG, with the protection of nitrogen. Stannous octoate dissolved in dry toluene was transferred into the flask [Stannous octoate/lactide, 0.15 % (w/w)]. The mixture solution was heated to 130 °C and allowed to reflux for 12 h (Scheme 1). The products were purified by repeated dissolution into dichloromethane and precipitated by cold ethanol. The isolated polymers were dried at 40 °C under vacuum for 24 h. Various PLA–PEG–PLA triblock copolymers with different compositions were prepared using the same procedure. The copolymers characterized by GPC and ^1H NMR.

Micellar dispersions

Direct dissolution

Stock solutions were prepared directly by dissolving a given amount of copolymer (P_1, P_2, P_3, and P_4) in phosphate-buffered saline (PBS), pH 7.4 (10–15 mg/mL). Samples were stirred for 30 min at room temperature (RT), and then sonicated 15 min to obtain a homogenized dispersion. Desired concentrations were prepared by dilution of the stock solutions.

Solvent injection

A solution of P_5 copolymer in DMF (15 mg/mL) was added dropwise to stirring buffer (PBS) to produce 0.3 mg/mL dispersion. The micellar dispersion was further stirred and then sonicated. Dilution of this stock solution was performed with diluting buffer, containing the same amount of DMF as the stock solution.

Critical association concentration (CAC)

ANS was used as a hydrophobic fluorescent probe. A given amount of the ANS stock solution in PBS (2 mM) was added to each of a series of the copolymer dispersions, followed by adding different amounts of buffer. The samples obtained, containing a fixed concentration (1.0×10^{-4} M) of ANS and varying concentrations (0.003–2.8 mg/mL; P5: 0.0005–0.28 mg/mL) of the copolymer, were stirred for 2 h at RT to achieve equilibration. Fluorescence measurements were performed at RT. Each spectrum was scanned three times to acquire the final fluorescence emission spectra.

Results and discussion

Copolymer characterization

Figure 1 shows the typical ^1H NMR spectrum of P_3. The peaks of CH_3 and CH in PLA blocks appear at 1.6 and

5.2 ppm, respectively, and the peak of main chain methylene units in PEG blocks is shown at 3.7 ppm. The α-methylene protons of PEG connected with PLA is clearly present at 4.3 ppm, indicating that the copolymerization of LA and PEG really takes place. The result corresponds to other reports on PLA/PEG block copolymers [3–5]. GPC measurements show monomodal molecular weight distributions, implying that no homopolymeric fractions of both PLA and PEG are present in products (not shown). Table 1 summarizes the molecular characteristics of triblock copolymers. It can be seen that copolymers with different molecular weights and compositions can be obtained by copolymerizing LA and PEG under controlled conditions. The number average molecular weight of the copolymers fell into the range of 4,700–50,000. The polydispersity indices (PDI = M_w/M_n) of the copolymers P_2, P_3, and P_4 are inferior to 1.4. It indicates the narrow molecular weight distribution of these copolymers, which is a critical factor for forming nanoparticles with similar structures.

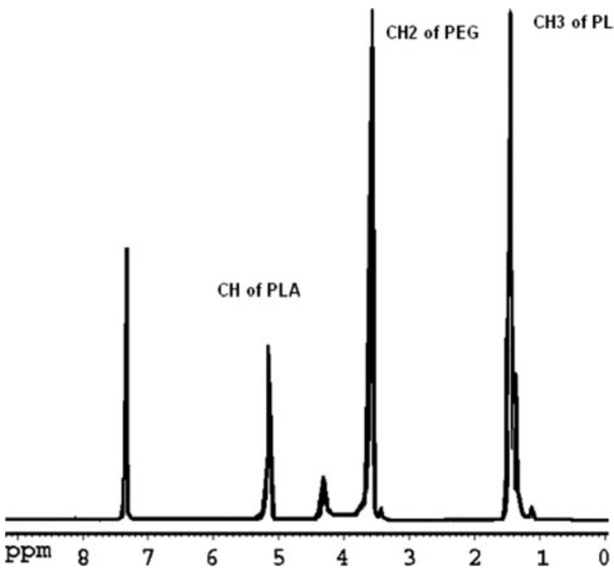


Fig. 1 ^{1}H NMR spectrum of P_3 copolymer, in $CDCl_3$

CAC determination

One of the usual ways to measure the critical association concentration (CAC) of copolymers is by the fluorescence probe technique. Here, ANS was employed as a fluorescent probe. Changes in ANS fluorescence on micelle formation are used to establish the CAC, which is a measure of the stability of the associates [2]. Typical emission spectra of ANS in aqueous solutions of P_3 copolymer at various concentrations are shown in Fig. 2a. The two noteworthy features of these spectra are that the fluorescence intensity shows a marked enhancement with increasing copolymer concentration, and that there is a progressive shift in the emission maximum toward the blue, from about 532–488 nm. These changes are due to the transfer of ANS molecules from the aqueous environment to the hydrophobic parts of the micellar structures and thus provide information on the location of ANS probe in the system [6].

Figure 2b shows the fluorescence intensity at λ_{max} as a function of P_3 copolymer concentration. It is observed that the intensity has a negligible change below a certain concentration and increases substantially thereafter. The CAC is taken as the intersection of the two straight lines drawn through the points at the lower copolymer concentrations, lying on a nearly horizontal line, and the points on the rapidly rising part of the plot. This indicates a CAC of about 20 μM. Table 1 lists the CAC values of the five triblocks. The copolymers have low CAC values, which indicate a good stability of the micelles in aqueous solutions. The values also vary with block lengths. Figure 3 shows the logarithmic CAC values versus the total LA monomer units in a polymer chain. The data points are basically on the straight line ($R^2 = 0.95$). The longer the LA block length, the smaller the CAC is.

Similar studies have been performed on some surfactants in aqueous solutions. They have reported that both blocks could influence the CAC value with the hydrophobic block having a strong negative effect and the hydrophilic block having only a minor positive effect [7]. In our case, a comparison of the data points of copolymer

Table 1 Molecular characteristics of PLA–PEG–PLA triblock copolymers and their CAC values in aqueous solutions

Number	M_{nPEG}	^{1}H NMR		GPC		CAC × 10^6 (mol/L)
		Block ratio (LA/EO) in product	Copolymer[a]	M_n	M_w/M_n	
P_1	3,000	0.44	LA_{15}–EO_{68}–LA_{15}	4,750	1.7	150.3
P_2	3,000	1.91	LA_{65}–EO_{68}–LA_{65}	12,406	1.26	42.3
P_3	4,000	2.90	LA_{132}–EO_{91}–LA_{132}	22,994	1.18	20
P_4	6,000	0.54	LA_{37}–EO_{136}–LA_{37}	14,540	1.33	46.5
P_5	6,000	5.28	LA_{359}–EO_{136}–LA_{359}	49,631	1.77	2

[a] Copolymers are denoted by lactic acid–ethylene oxide–lactic acid followed by the degrees of polymerization of the blocks

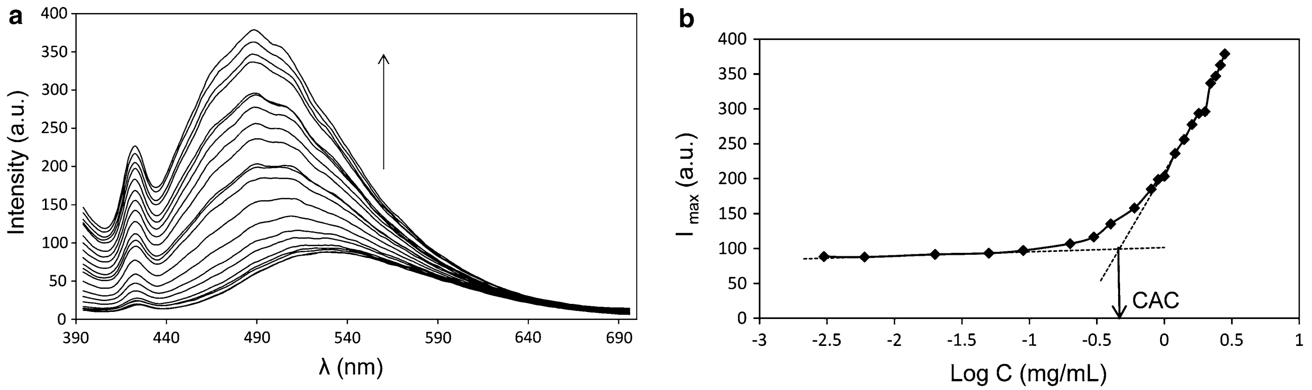


Fig. 2 **a** Fluorescence spectra of ANS in PBS with increasing concentrations of P_3 copolymer (*arrow direction*). Excitation and emission slits: 10.0 nm, scan rate: 600 nm/min, $\lambda_{ex} = 370$ nm, 25 °C. **b** Plot of the fluorescence intensity at λ_{max} versus the P_3 concentration

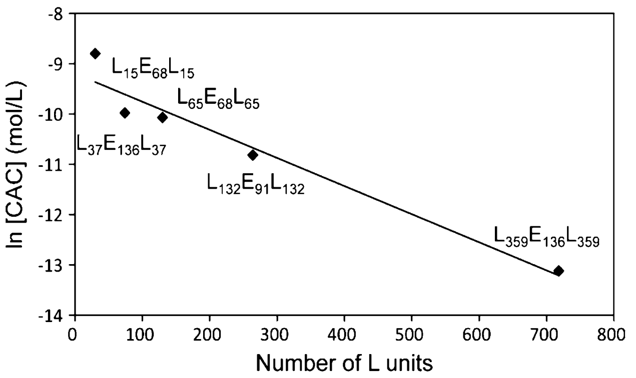


Fig. 3 Plot of logarithmic CAC values of PLA–PEG–PLA triblock copolymers in aqueous solution versus number of LA units in a polymer chain at 25 °C. The data points were labeled as $L_nE_mL_n$. *L* lactic acid, *E* ethylene oxide

LA_{15}–EO_{68}–LA_{15} with LA_{37}–EO_{136}–LA_{37}, and also of other points, indicates that the CAC of PLA–PEG–PLA triblock copolymers is mainly determined by the length of the hydrophobic LA block, whereas the length of the EO block has little effect. This result is in good agreement with the findings of Liu et al. [7] on oxyethylene/oxybutylene ($B_nE_mB_n$) triblock copolymers in aqueous solution. Other studies have also been performed on PEG/PLA block copolymers having a fixed length of PEG, and the effect of PLA block on CAC was confirmed [3]. In the present study, triblock copolymers have a large difference in the EO block length (from 68 to 136), which gives us the opportunity to summarize the results more effectively.

Conclusion

The self-assembly of PLA–PEG–PLA triblock copolymers in aqueous solutions was studied by the fluorescence probe technique. The results show that the length of the hydrophobic end LA blocks is the dominant factor on the CAC values of the micelles. Increasing the LA block length enhances the hydrophobicity of the copolymer chains. Thus, they can self-assemble more easily to form micelles, leading to lower CAC values. The effect of the EO block length is insignificant. Furthermore, the results show that the copolymers have low CAC values indicating the desirable stability of the micelles.

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