SELF-ASSEMBLING MICELLES OBTAINED FROM PLLA/PEG AND PDLA/PEG BLOCK COPOLYMERS IN AQUEOUS SOLUTIONS

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Abstract

A series of polylactide-poly(ethylene glycol) (PLA-PEG) block copolymers were synthesized by ring-opening polymerization of L- or D-lactide in the presence of mono- or dihydroxyl PEG, using nontoxic zinc lactate as catalyst. Micelles were then prepared by direct dissolution of the obtained copolymers in aqueous medium without heating or using any organic solvents. Aqueous gel permeation chromatography and dynamic light scattering measurements were carried out to characterize the resulting micelles. Generally, mixed micelles containing both PLLA/PEG and PDLA/PEG copolymers appear lager and more compact compared to single ones. However, the size of mixed micelles is smaller than that of single ones which exhibit an anisotropic structure since stereocomplexation disfavors the formation of anisotropic micelles. The copolymer parameters such as structures, molar mass and PEG fraction strongly influence the formation of anisotropic micelles, and thus lead to various micellar sizes.

Keywords: *polylactide, poly(ethylene glycol), stereocomplexation, self-assembly, anisotropy*

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Introduction

In the past decades, nanoparticles, micelles and vesicles prepared by self-assembly of amphiphilic copolymers have been widely investigated for applications in the field of sustained drug delivery [1]. Compared to conventional drug administration routes, these drug delivery systems (DDS) present numerous advantages such as constant blood drug concentration, reduced drug dosage, decreased drug administration frequency, reduced side effects, etc. Among all the polymers used for DDS, polylactide/poly(ethylene glycol) (PLA/PEG) copolymers appear the most promising due to their outstanding properties [2]. In fact, PLA exhibits good biocompatibility and degradability, while PEG is well soluble in water and in most organic solvents, non-toxic and can be eliminated through kidney filtration when the molar mass is below 30000.

Stereocomplexation between PLLA and PDLA was found to improve the properties of PLA/PEG micelles [3,4]. Kang et al. reported that the "stereocomplex-type" micelles derived from PLLA/PEG and PDLA/PEG copolymers exhibit higher aggregation number, smaller volume, and more compact structure compared to single micelles [3]. However, our previous work showed that the average size of mixed micelles is smaller than single micelles for some copolymers [4]. On the other hand, aqueous gel permeation chromatography (GPC) allowed to identity both peaks of micelles and free copolymers, and to determine the aggregation number of micelles [5]. Therefore, it is of great interest to comparatively investigate the properties of single and mixed micelles.

In this work, a series of PLA/PEG block copolymers were synthesized and characterized. Self-assembling micelles by direct dissolution in water were investigated by aqueous GPC, dynamic light scattering and transmission electron microscopy (TEM), taking into account the effects of stereocomplexation between PLLA and PDLA blocks for different copolymers.

Materials and methods

L-lactide and D-lactide were obtained from Purac and purified by crystallization from ethyl acetate. Monomethoxy poly(ethylene glycol) (mPEG) with molar masses of 2000 and 5000 and dihydroxyl PEG with molar masses of 2000, 4000 and 8000 were supplied by Fluka. Zinc lactate was purchased from Sigma. All organic solvents were of analytic grade and used without further purification.

PLA/PEG block copolymers were synthesized by ring-opening polymerization as described previously. Briefly, predetermined amounts of PEG and L- or D-lactide were introduced in a polymerization ampoule, the initial molar ratio of ethylene oxide to lactyl repeat units (EO/LA) ranging from 2 to 6. Zinc lactate (0.1 wt%) was then added. After degassing, the ampoule was sealed under vacuum, and polymerization was allowed to proceed at 130°C for 3 days. After that, the product was recovered by dissolution in dichloromethane and precipitation in diethyl ether. The product was finally dried under vacuum up to constant weight.

Predetermined amounts of PLLA/PEG, PDLA/PEG or their equal molar mixtures were dissolved in distilled water under stirring at room temperature for 2 hours, yielding homogeneous micellar solutions with different concentrations.

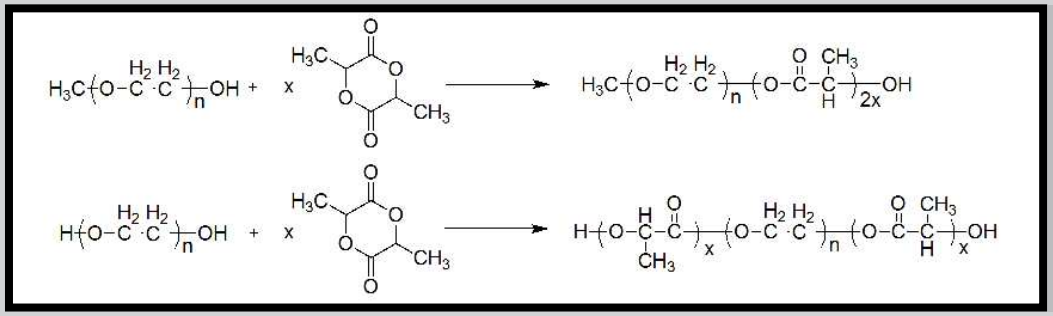
Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded at room temperature with a Bruker spectrometer operating at 250 MHz by using CDCl3 as solvent. The relaxation delay used for ^{1}H-NMR spectra was 800 msec, and chemical shifts (δ) were given in ppm using tetramethylsilane as an internal reference.

Gel permeation chromatography (GPC) measurements in organic solvent were performed on a Waters 410 apparatus equipped with a RI detector. THF was used as the mobile phase at a flow rate of 1.0 ml/min. All the solutions were prepared at a concentration of 10 g/L and filtered through 0.22 μm Millipore filters. 20 μl of solution were injected for each analysis. Calibration was accomplished with polystyrene standards (Polysciences, Warrington, PA).

GPC measurements in aqueous medium were carried out with a series of 3 columns, PL aquagel-OH 30, 40 and 50, Polymer Laboratories Corporation, connected with an RI detector. Water/methanol (80:20, v/v) was used as the eluent at a flow rate of 1.0 ml/min at room temperature. PEG was used as standards for the calibration. All the solutions were prepared at a concentration of 5 g/L and filtered through 0.45 μm Millipore membrane filters before injection.

Dynamic light scattering (DLS) was carried out on a Sympatec Nanophox equipment with vertically polarized incident light of wavelength λ = 632.8 nm supplied by a HeNe-Laser operating at 10 mW max. Measurements were made at 25°C and at an angle of 90°. All the solutions were filtered through 0.80 μm Millipore membrane filters. The autocorrelation functions from DLS were analyzed by using the photon cross correlation spectroscopy (PCCS) method to obtain the diameter distributions.

TEM was performed on a Hitachi H-600 electron microscope, operating at an accelerating voltage of 75 kV. One drop of micelle solution was placed on a copper grid covered with nitrocellulose membrane and air dried before measurement.



SCHEME 1. Ring opening polymerization of L- or D-lactide in the presence of mono- and dihydroxyl PEG.

Results and Discussions

Both diblock and triblock PLA/PEG copolymers were synthesized by ring-opening polymerization of L- or D-lactide in the presence of mono- or dihydroxyl PEG (SCHEME 1). The reaction was performed at 130°C for 3 days. Zinc lactate was used as catalyst instead of stannous octoate or other catalysts which are more or less cytotoxic. The yield of the reactions ranged from 80 to 90%.

TABLE 1 presents the molecular characteristics of the resulting copolymers, including number average molar mass (Mn), molar mass distribution (D=Mw/Mn), and copolymer composition as determined by using ^{1}H NMR and GPC. ^{1}H NMR measurements allowed to determine the composition of various PLA/PEG copolymers from the integrations of NMR resonances belonging to the methylene protons of ethylene oxide units of PEG at 3.6 ppm and to the methine proton of lactyl units of PLA at 5.2 ppm. For the sake of clarity, the triblock copolymers are named as LxEOyLx or DxEOyDx, and the diblock copolymers as EOyLx or EOyDx. In these acronyms, L, D, and EO represent PLLA, PDLA, and PEG blocks, respectively, x and y representing the number-average degree of polymerization of corresponding blocks. As reported in our previous work, the EO/LA ratio of the copolymers was found to be higher than the feed ratio because the conversion of lactide was not complete, and unreacted lactide was eliminated by the purification procedure [6].The polydispersity index is inferior to 1.2 for all copolymers, in agreement with narrow molar mass distributions.

TABLE 1. Molecular characteristics of PLA/PEG copolymers.

Acronym	Copolymer	Mn PEG	EO/LA[a]	DP_{PEG}[b]	DP_{PLA}[c]	M_n[d]	M_n[e]	D[f]
L1	$EO_{45}L_{12}$	2000	3.85(3.0)[g]	45	12	2864	4860	1.18
D1	$EO_{45}D_{12}$	2000	3.80(3.0)	45	12	2864	4810	1.17
L2	$EO_{113}L_{32}$	5000	3.55(3.0)	113	32	7304	9110	1.11
D2	$EO_{113}D_{31}$	5000	3.63(3.0)	113	31	7232	9590	1.12
L3	$L_{10}EO_{45}L_{10}$	2000	2.23(2.0)	45	20	3440	5510	1.13
D3	$D_{11}EO_{45}D_{11}$	2000	2.12(2.0)	45	21	3512	5630	1.15
L4	$L_{12}EO_{91}L_{12}$	4000	3.75(3.0)	91	24	5728	8460	1.10
D4	$D_{11}EO_{91}D_{11}$	4000	4.10(3.0)	91	22	5584	8380	1.08

[a] Calculated from the integration of NMR bands belonging to PEG block at 3.6 ppm and to PLA block at 5.2 ppm.
[b] $DP_{PEG}=M_{nPEG}/44$.
[c] $DP_{PLA}=DP_{PEG}/(EO/LA)$.
[d] $M_n=M_{nPEG}+DP_{PLA}\cdot 72$.
[e] Determined by GPC.
[f] Polydispersity index, determined by GPC.
[g] Date in parentheses represent the EO/LA ratio in feed.

Aqueous GPC was employed to evaluate the average size of micelles (FIG. 1). Two peaks are observed on the GPC curves of both D1 and LD1 micelles: the peak at long elution time is assigned to free copolymer chains, while the one at short elution time belongs to the micelles. The peak at long elution time appears at 26.5 min for both solutions because the two copolymers have the same molar mass (TABLE 1). In contrast, the two samples present different elution times for micelles: the peak of D1 is detected at 13.6 min, and that of LD1 at 14.4 min. This indicates that the size of D1 micelles is larger than that of LD1 ones although the absolute molar masses cannot be determined as the peaks are beyond the calibration range. In contrast, the situation seems different in the case of D2 and LD2 micelles as shown in FIG. 1b. In fact, the peak of D2 micelles is detected at 14.4 min, and that of LD2 micelles at 13.9 min, while the peaks of free copolymers appearing at the same elution time, i.e. 25.3 min. Similar results were also found in D3/LD3 and D4/LD4 samples, which indicates that the size of mixed micelles is larger than that of single ones.

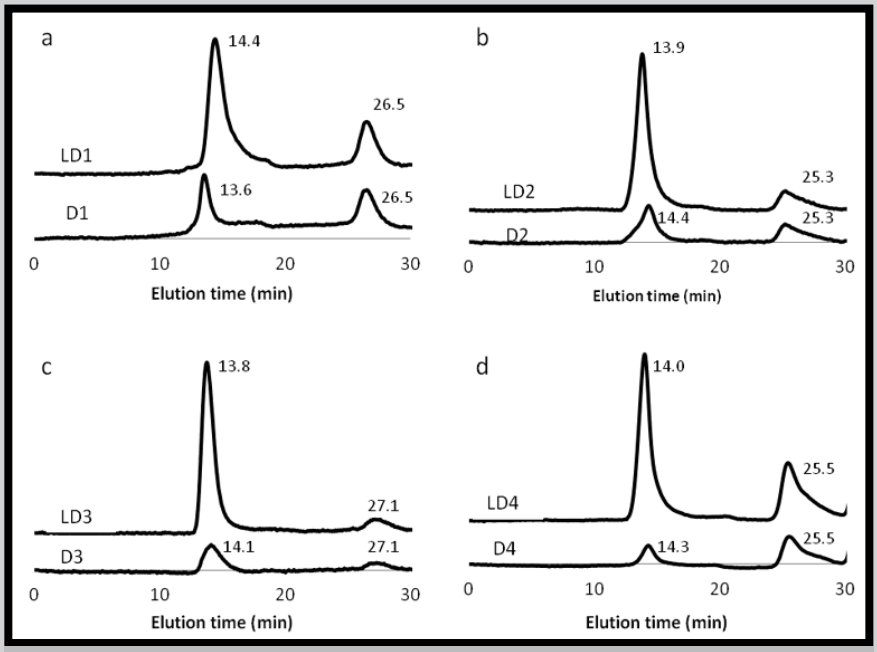


FIG. 1. Aqueous GPC chromatograms of single and mixed micelles at 5 g/l: a) D1 and LD1, b) D2 and LD2, c) D3 and LD3, d) D4 and LD4.

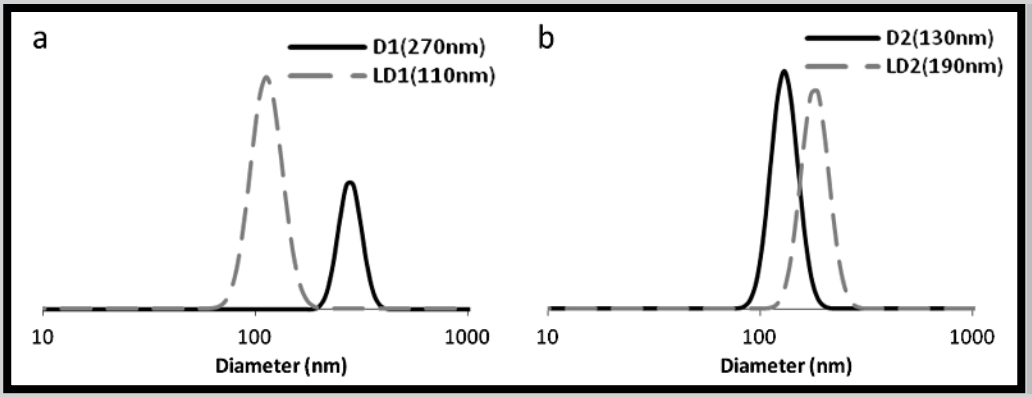


FIG. 2. DLS grafts of single and mixed micelles at 5 g/l: a) D1 and LD1, b) D2 and LD2.

The micellar size was determined by DLS measurements in comparison with aqueous GPC results. FIG. 2 shows that the size decreases from 270 nm for D1 to 110 nm for LD1 micelles, but increases from 130 nm for D2 to 190 nm for LD2 micelles. Therefore, the same size variation tendency was found in both aqueous GPC and DLS measurements. The size of mixed micelles is larger than single ones for most compolymers, except in the case of D1/LD1 micelles.

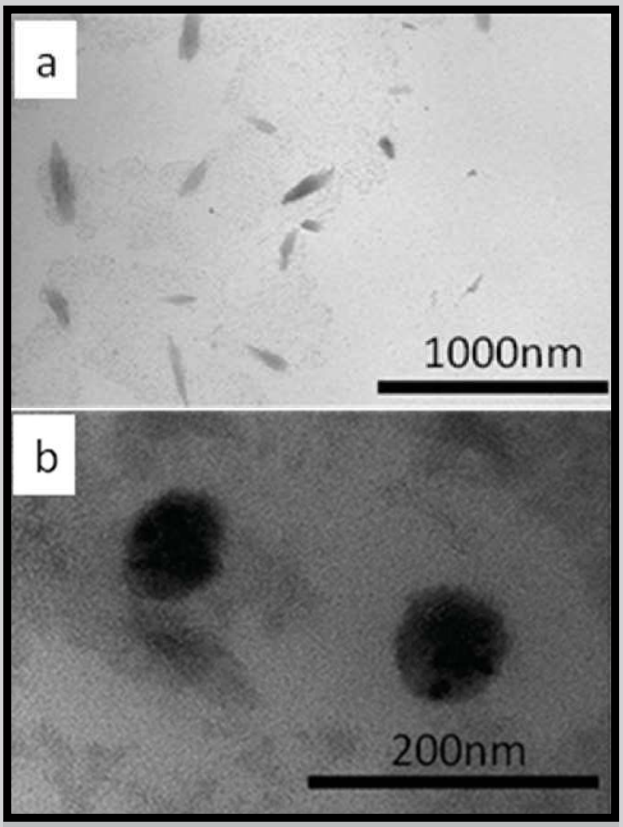


FIG. 3. TEM micrographs of single and mixed micelles at 5 g/l: a) D1 micelles, b) LD1 micelles.

TEM measurements were then carried out to examine the morphology of the micelles. According to previously reported literature data, all the copolymers in this work are supposed to form spherical micelles in aqueous solution due to high PEO fraction [7]. Interestingly, large anisotropic micelles ware found for D1 sample, while all other samples exhibit spherical structures (FIG. 3). The formation of anisotropic micelles well explained the different size variations of micelles for all the copolymers. In fact, stereocomplexation leads to larger size and more compact structure in the case of spherical micelles, as Kang et al. reported previously [3]. On the other hand, stereocomplexation disfavors the formation of anisotropic micelles [8]. Therefore, for the copolymers able to form anisotropic micelles, mixed micelles appear smaller compared to single ones. The formation of anisotropic micelles depends on the copolymer parameters such as PEG fraction, molar mass, stereocomplexation, etc, as reported in our previous work [8].

Conclusions

The variation of micellar size as a consequence of stereocomlaxetion was investigated for a series of copolymers with different structures, molar masses and PEG fractions. Both aqueous GPC and DLS measurements show that the size of mixed micelles is larger than single ones for most copolymers. In the case of D1 and LD1 micelles, however, opposite behavior was observed, DL1 mixed micelles appearing larger than D1 single ones. This difference is well explained by the formation of anisotropic micelles in the case of D1 copolymer as shown in TEM images. Therefore, stereocomplexation leads to a larger size and more compact structure of spherical micelles, as reported previously [3]. However, the size of mixed micelles is smaller than that of single ones which exhibit an anisotropic structure since stereocomplexation disfavors the formation of anisotropic micelles as reported in our previous work [8]. The copolymer parameters such as structures, molar mass and PEG fraction strongly influence the formation of anisotropic micelles, and thus lead to various micellar sizes.

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