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Notes

6-Arm PLLA-PEG Block Copolymers for Micelle Formation and Controlled Drug Release

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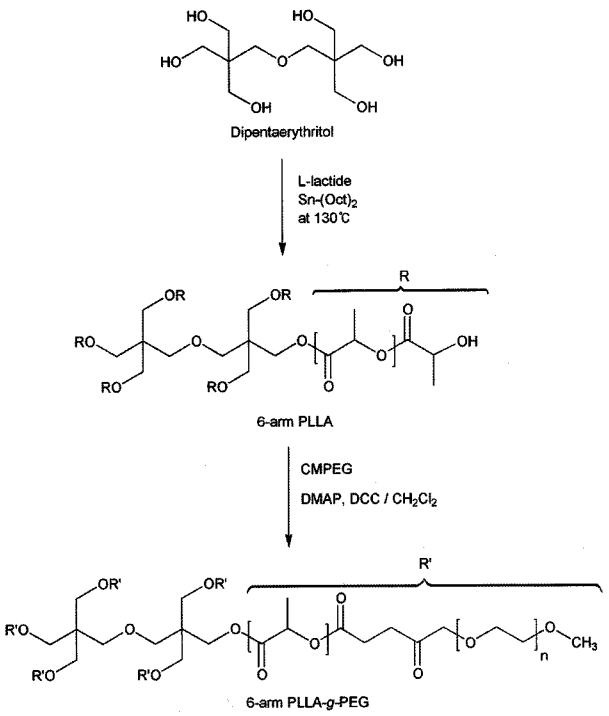
Introduction

For the last decade, multi-arm, or called star-shaped, polymers have attracted attention to utilize their various block polymers as hydrogels or micelles in biomedical fields, mainly drug delivery systems.[1,2] The reason of such attention is that multi-arm polymer shows smaller hydrodynamic size and lower solution viscosity as compared with linear polymer, resulting in complete renal excretion after degradation in the body.[1] In particular, multi-arm poly(ether-ester)s have been mainly investigated to apply to many applications as substitutes of Pluronic due to their thermo-reversible and biodegradable properties.[3-5] Choi *et al.* synthesized multi-arm PEO-PLA and PEO-PCL block copolymers with four kinds of the arm number and characterized their physical properties.[6] They showed that the degree of swelling in aqueous solution and phase separation were changed by varying the number of the arm, confirming usefulness for controlled delivery of drug. Li *et al.* synthesized multi-arm PEO-PLGA block copolymers and tested *in vitro* degradation of the polymers.[7] In their study, four- and eight-arm chains exhibited different degradation properties due to their different molecular architectures as compared with linear chain. Park *et al.* synthesized multi-arm PLLA-PEO block copolymers.[1,8] Their studies focused on solution properties of the polymers to investigate sol-gel transition behavior and micellization. Lee *et al.* also synthesized multi-arm PLGA-PEG block copolymers and investigated their sol-gel transition behavior and micellization.[9] In their studies, they investigated the effect of temperature and concentration on micellization and sol-gel transition behavior. But, unfortunately, further studies on applications of drug delivery system have not been performed. According to many previous reports, polymeric micelle has many advantages for drug delivery system because of their unique characteristics in the body, such as dissolving hydrophobic molecules, thermodynamic stability, drug release for an extended time and repressed rapid clearance by the RES due to their size and surface characteristics.[10-12] So, we were interested in studying release behaviors of drug using micelles of degradable multi-arm copolymers. Particularly, it was expected that the polyester chains to construct the core of micelle play key role of controlling release behavior of encapsulated drug.

Herein, we report on the micellization of novel 6-arm PLLA-PEG copolymers and the controlled release behavior of drug from the micelle. In detail, we discuss about the effect of chain lengths of the 6-arm PLLA-PEG on micelle formation and release rate of drug.

Experimental

6-Arm PLLA-PEG copolymers were synthesized via a simple route as shown in Scheme I. The 6-arm PLLAs were synthesized by bulk ring-opening polymerization of L-lactide with dipentaerythritol as a 6-arm initiator and stannous octoate (St-Oct) as a catalyst. Briefly, Pure L-lactide (15 g,



Scheme I. Synthetic route of 6-arm PLLA-PEG.

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104.1 mmol) and dipentaerythritol (1.5 g, 12.4 mmol) were placed in a dried glass ampoule containing a Teflon-coated magnetic stirring bar and St-Oct (0.21 g, 0.52 mmol) was added as a catalyst. The mixture was stirred under vacuum at 130 °C for 6 h, followed by processes containing dissolving in chloroform, filtering, precipitating in *n*-hexane, and drying under vacuum. The molar ratio of L-lactide to St-Oct was fixed with 200 according to references.[13,14] Carboxymethyl mono-methoxy poly(ethylene glycol) (CMPEG) was synthesized like a report.[15] Then, the prepared CMPEG was conjugated to the 6-arm PLLA to give 6-arm PLLA-PEG. 6-Arm PLLA (5.5 g, 1.0 mmol), CMPEG (12.1 g, 6.04 mmol), DCC (1.25 g, 6.04 mmol) and DMAP (0.15 g, 1.21 mmol) were dissolved in 200 mL of anhydrous methylene chloride and stirred at room temperature for 48 h under N_2 atmosphrere. The reaction mixtures were treated by several processes containing filtration, crystallization and dialysis to yield powdery compound.[8] The structure of synthesized 6-arm PLLA, CMPEG, 6-arm PLLA-PEG was confirmed by FT-IR (Nicolet Magma-IR 550) and ^{1}H-NMR (400 MHz, Varian). GPC (M616LC System, Waters) was used to obtain the molecular weight and polydispersity index (PDI) of polymers.

Micelles incorporating IMC were prepared with 6-arm PLLA-PEG by the solvent evaporation method as follows. First, 6-arm PLLA-PEGs and IMC were dissolved in distilled water and ethanol, respectively. Second, the IMC solution was added to 6-arm PLLA-PEG solution. Finally, the mixture solution was shaken at 37 °C, 50 rpm for 24 h, followed by drying overnight at 60 °C. The residual IMC was completely removed by centrifugation (4,000 rpm, 10 min) and the separated micelle solution was sonicated and freeze-dried. The amount of IMC loaded in the micelles was determined by measuring the UV absorbance of IMC at 318 nm and the loading efficiency (LE) was calculated by the weight ratio of the measured IMC to IMC-loaded micelles. The critical micelle concentration (CMC) of micelles was analyzed with pyrene as a probe by fluorescence measurements (FP-6500, JASCO).[16] The diameter and the size distribution of micelles were measured by dynamic light scattering (DLS) analyzer (FPAR-1000, Photal). The intensity autocorrelation data was measured at the scattering angle of 160° at 25 °C for 120 s.

The release behavior of IMC from micelles was observed by following procedures. The IMC loaded into micelles was released in 0.01 M phosphate buffered saline solution (PBS, pH 7.4) at 37 °C for 35 days. At determined times, the released IMC in the micelle solution was determined and fresh PBS solution was added.

Results and Discussion

^{1}H-NMR spectra provided structural information of synthesized polymers. The characteristic peaks for the 6-arm PLLA chain were found at 1.55 and 5.1 ppm, respectively, indicating successful polymerization of L-lactide. And, two peaks assigned to methylene proton at dipentaerythritol were observed at 3.28 and 4.16 ppm. These signals provided structural information to estimate the molecular weight of the synthesized 6-arm PLLAs, by comparing the ratio of peak areas. The PEG conjugation to 6-arm PLLA was confirmed by the disappearance of signal for the methylene proton of PEG that is adjacent to the ester linkage at 4.23 ppm (-OCH_2 $\underline{CH_2}$COO-). FT-IR spectra of 6-arm PLLA, 6-arm PLLA-PEG and CMPEG were shown in Figure 1. As shown in Figure 1(a), the 6-arm PLLA-PEG showed the distinct carbonyl stretching bands for the PLLA block at ~1750 cm^{-1}, which was not seen in Figure 1(c) for CMPEG, and the intense bands of methylene group for the PEG block at ~2900 cm^{-1}, which was not in Figure 1(b) for 6-arm PLLA. Also, the 6-arm PLLA-PEG presented two attenuated peaks that are assigned to C-H bending signal of PEG segment at around 1560 and 1630 cm^{-1}. The signals were not seen in the Figure 1(b) indicating 6-arm PLLA and strongly appeared in the Figure 1(c) for CMPEG. The molecular weight of polymers that was obtained by GPC was listed in Table I. The series of 6-arm PLLAs with different molecular

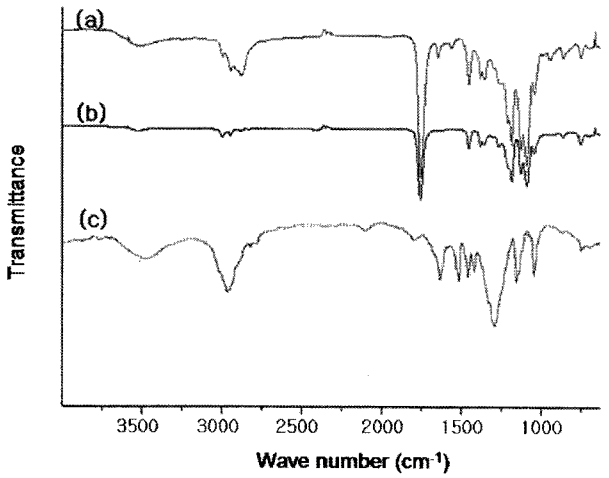

Figure 1. FT-IR spectra of (a) 6-arm PLLA-PEG, (b) 6-arm PLLA, and (c) CMPEG.

Table I. The Molecular Weight and the PDI of 6-Arm PLLAs and 6-Arm PLLA-PEGs

Polymers	M_n	M_w	PDI[a]
6-Arm PLLA 3.8 K	3,800	4,700	1.24
6-Arm PLLA 4.5 K	4,500	5,500	1.24
6-Arm PLLA 6 K	6,000	7,500	1.25
6-Arm PLLA 3.8 K-PEG 2 K	13,600	17,000	1.32
6-Arm PLLA 4.5 K-PEG 2 K	14,500	19,400	1.34
6-Arm PLLA 6 K-PEG 2 K	16,800	22,700	1.35
6-Arm PLLA 4.5 K-PEG 5 K	29,000	43,500	1.50

[a] Polydispersity index (M_w/M_n).

Table II. Physical Properties and Drug Loading of 6-Arm PLLA-PEG Micelles

Polymers	CMC (g/L)	Size (nm)		DLA (μg/mg)	DLE (%)
		Pre-Loaded	IMC-Loaded		
6-Arm 3.8 k-PEG2k	0.151	180.4 ± 39.4	117.4 ± 19.7	4.2 ± 0.1	42.4 ± 0.9
6-Arm 4.5 k-PEG2k	0.076	103.4 ± 6.2	90.2 ± 1.1	5.5 ± 0.1	54.5 ± 1.4
6-Arm 6.0 k-PEG2k	0.042	190.4 ± 7.9	158.0 ± 5.4	5.4 ± 0.1	53.9 ± 0.4
6-Arm 4.5 k-PEG5k	0.083	125.9 ± 12.3	94.4 ± 5.2	5.6 ± 0.3	56.3 ± 3.5

weights was synthesized and presented the molecular weight as 3,800, 4,500, and 6,000, showing almost similar PDIs of 1.24. The conjugation of CMPEG to each 6-arm PLLA gave three kinds of 6-arm PLLA-PEG, showing almost same PDIs at the range of 1.32-1.35. The molecular weight data indicates that the polymerization of L-lactide for 6-arm PLLA and the conjugation of PEG to the 6-arm PLLA were well performed as expected. But, the PDI of 6-arm PLLA-PEG was little higher than that of 6-arm PLLA. This means that the molecular weight distribution of 6-arm PLLA-PEGs was little dispersed after the conjugation of PEG, comparing with 6-arm PLLA. This result can be demonstrated that the 6-arm PLLA4.5K-PEG5K conjugated with longer CMPEG showed the higher PDI.

The CMC data was listed up in Table II. The CMC values decreased as increasing the molecular weight of PLLA, in which the length of PEG chains was 2,000. This result indicates that more hydrophobic chains are energetically effective for the micelle formation, which is consistent with cases of Pluronic and other amphiphilic star-shaped copolymers.[17] This explanation also demonstrates that the 6-arm PLLA-PEG containing longer PEG chain (6-arm PLLA4.5K-PEG5K) showed higher CMC value than those with shorter PEG chain (6-arm PLLA4.5K-PEG2K). The size of micelles

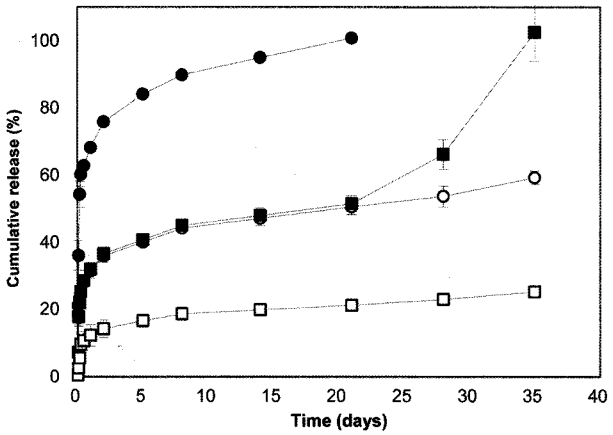

Figure 2. The release profile of IMC from 6-arm PLLA-PEG micelles. PLLA 3.8K-PEG2K (closed circles), PLLA4.5K-PEG2K (opened circles), PLLA4.5K-PEG5K (closed squares), and PLLA6K-PEG2K (opened squares).

was revealed between about 100-200 nm. In particular, the hydrodynamic sizes (the diameter of micelles) decreased after encapsulating IMC, indicating that the incorporation of IMC into the hydrophobic core of micelle structure makes smaller structure during micellization. The loading amounts and efficiencies of IMC were also shown in Table II. These had a tendency to show higher values at more hydrophobic chains. This tendency is because the hydrophobic IMC is more favorable to incorporate into larger space of the hydrophobic core of micelles.

The IMC release profiles of 6-arm PLLA-PEGs are shown in Figure 2. The profiles presented that the more hydrophobic 6-arm copolymers with longer PLLA chains distinctly slowly released the loaded IMC at the condition of the same PEG chain. This indicates that hydrophobic PLLA chain plays an important role in releasing the loaded IMC. It can be concluded that the hydrophobic PLLA retards the release of IMC from the hydrophobic core that is mainly constructed with PLLA. More interestingly, out of two copolymers with the same PLLA chain length, one containing PEG5K showed the drastic conversion of cumulative release percentage toward high level at the middle of release period, contrary to that with PEG2K. This can be explained with the hydrophilicity of PEG chain at aqueous solution. The hydrophilic PEG chains construct the outer shell of micelle structure, which are hydrated under aqueous environment.

Conclusions

A series of novel 6-arm PLLA-PEG copolymers was synthesized for drug delivery system. This block copolymer was prepared by conjugating PEG to 6-arm PLLA. The 6-arm PLLA-PEGs formed micelle at aqueous solution, showing different sizes and loading efficiency of IMC by changing PLLA chain length. Overall release profiles of IMC from 6-arm PLLA-PEG micelles showed no initial burst and prolonged release time. Particularly, 6-arm PLLA-PEG micelles with longer PLLA chain showed more sustained release behaviors. The release rate of IMC can be modulated with the chain length of PLLA.

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