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Short communication

Preparation of a polysaccharide–polyester diblock copolymer and its micellar characteristics

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Abstract

A novel amphiphilic block copolymer based on dextran and poly(ε-caprolactone) was synthesized, and characterized by ^{1}H NMR spectra. Its micellar characteristics in aqueous solution were investigated by fluorescence technique, transmission electron microscopy and dynamic light scattering. It was found that such polysaccharide derivative could self-assembly in water into spherical micelles with the diameters ranged from 20 to 50 nm in the absence of organic solvent or surfactant.

Keywords: Dextran; Polycaprolactone; Amphiphilic block copolymer; Micellar characteristics

1. Introduction

Amphiphilic block copolymers, which consist of hydrophilic and hydrophobic segments, and their self-assembly in aqueous solutions are currently a topic of great interest (Alli, Hazer, Menceloğlu, & Süzer, 2006; Chen, Yu, Cheng, Yu, & Cheung, 2006; Dimitrov et al., 2006; Jang et al., 2006; Jung & Yoshida, 2006; Lei & Fan, 2006; Trimaille, Mondon, Gurny, & Möller, 2006; Zhang, Grijpma, & Feijen, 2006). This interest is mainly motivated by their attractive applications to various research areas such as detergents, surface coating, oil recovery, drug delivery carrier technology and nanotechnology. In particular, various block copolymers consisting of poly(ethylene glycol) (PEG) and biodegradable polyesters such as poly(L-lactic acid) (PLLA), poly(glycolic acid) (PGA), or their copolyesters (PLGA) have been prepared, and their self-assembled micelles have found very important uses in the biomedical materials field (Cai, Bei, & Wang, 2002; Cho, Lee, Lee, Huh, & Park, 2004; Deschamps et al., 2004; Yamamoto, Yasugi, Harada, Nagasaki, & Kataoka, 2002; Zhou, Deng, & Yang, 2004). However, one drawback of these PEG-based block copolymers is the absence of reactive groups at their molecular chains, which limits further modification or ligand coupling. In contrast, naturally occurring polysaccharides with good hydrophilicity, biocompatibility and biodegradability seem to be attractive alternatives to PEG hydrophilic segments for designing amphiphilic block copolymers. Up to now, however, only a few of studies have dealt with polysaccharide-based block copolymers. For example, Akiyoshi, Maruichi, Kohara, and Kitamura (2002) obtained the block copolymers of poly(ethylene oxide) and amylose by an enzymatic reaction; Loos and Stadler (1997) prepared the linear block copolymers of polystyrene and polysaccharide by a block synthesis method, and investigated their interfacial behavior; very recently, Yang, Kataoka, and Winnik (2005) reported on the synthesis of diblock copolymers consisting of hyaluronan and poly(2-ethyl-2-oxazoline), a pseudopeptide block; Kamitakahara and Nakatsubo (2005) reported on the preparation of diblock copolymers consisting of cellulose and a hydrophobic part, azidoalkyl carboxylic acid. To our knowledge, however, there is no information available on the amphiphilic block copolymers consisting of a polysaccharide segment and a biodegradable polyester segment.

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In this short communication, we reported the first synthesis of a novel amphiphilic block copolymer based on the combination of a natural polysaccharide (dextran) with a synthetic aliphatic polyester (poly(ε-caprolactone)) and its micellar characteristics in aqueous solution. In order to obtain such material, the poly(ε-caprolactone) end-capped with the acryloyl group and the amino-functionalized dextran were prepared, respectively, and the subsequent coupling reaction was carried out.

2. Experimental

2.1. Materials

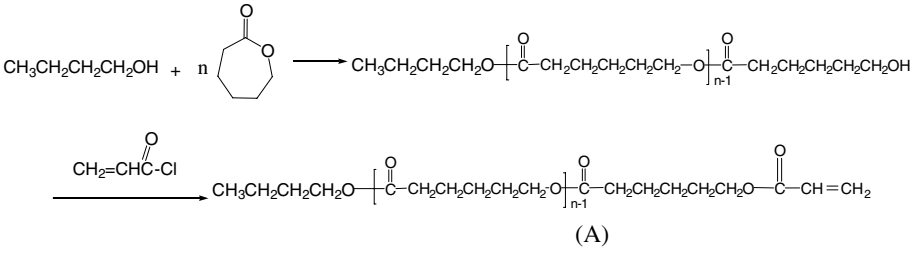
Dextran from Leuconostoc mesenteroides was obtained from Fluka, and its number average molecular weights (M_n) was determined to be 3700 g/mol by gel permeation chromatograph (GPC). ε-Caprolactone (CL) was purchased from Sigma (St. Louis, MO). Before the use, it was dried over CaH_2 for 24 h and distilled. Sodium cyanoborohydride was purchased from Acros. All other reagents were analytical grade and used as received.

2.2. Preparation of poly(ε-caprolactone) end-capped with acryloyl group

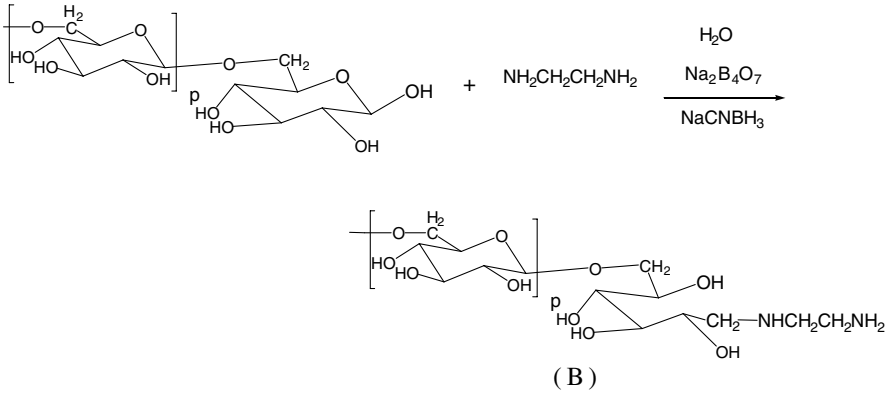
The poly(ε-caprolactone) end-capped with the acryloyl group (Product **A**) was synthesized according to Scheme 1. At first, the poly(ε-caprolactone) with hydoxyl end-group (PCL-OH) was obtained by the ring opening polymerization of ε-caprolactone (CL) in the presence of *n*-butyl alcohol. For this purpose, 20 g CL, 0.5 g *n*-butyl alcohol and 0.05 g stannous octanoate were put into an ampoule with magnetic stirrer connected to the vacuum/Ar line, and the polymerizations were carried out under Ar atmosphere at 120 °C in a silicone oil bath. After 5 h, the reaction was terminated by dipping the reaction flask into ice. The resulting PCL-OH was purified by four successive precipitation using THF as the solvent and methanol as the non-solvent, and dried at room temperature under vacuum for 24 h. To obtain the product **A**, 1.45 g triethylamine was added into a solution of PCL-OH in 150 ml dry DMF, followed by adding slowly 1.2 g acryloyl chloride within 40 min at 0 °C. After that, the temperature was raised to 25 °C and the reaction was conducted for 2 h. Then the reaction mixture was cooled to 0 °C and poured into methanol. The product **A** was recovered by filtration, purified by three successive precipitation using THF as the solvent and methanol as the non-solvent, and dried under vacuum. It has the average number molecular weight (M_n) of 2800 g/mol, as determined from ^{1}H NMR analysis.

2.3. Preparation of amino-functionalized dextran

The amino-functionalized dextran (Product **B**) was prepared according to Scheme 2. The dextran (5 g, M_n = 3700 g/mol), ethylenediamine (0.2 g) and aqueous sodium borate solution (100 ml, 0.1 mol/l) were added into the round-bottom reaction flask equipped with stirring, and the reaction was carried out at 60 °C for 8 days in a silicone



Scheme 1. Synthesis route of the polycaprolactone end-capped with the acryloyl group.



Scheme 2. Synthesis route of the amino-functionalized dextran.

oil bath. During the reaction, sodium cyanoborohydride (20 mg) was slowly added. After the reaction completion, the reaction mixture was poured into methanol. The precipitate was recovered by filtration, and then dried in vacuum at room temperature for 48 h. The obtained product **B** was purified by three successive precipitations using water as the solvent and methanol as the non-solvent.

2.4. Preparation of dextran-poly(ε-caprolactone) diblock copolymer

For the preparation of amphiphilic block copolymer based on dextran and poly(ε-caprolactone), the Micheal reaction between Products **A** and **B** was carried out, as indicated in Scheme 3. 1.12 g Product **A**, 2.00 g Product **B**, 50 mg hydroquinone, 80 mg *p*-methyl-benzene sulfonic acid, and 20 ml freshly distilled DMSO were added into a 50 ml round-bottomed three neck flask equipped with a magnetic stirrer. The coupling reaction was carried out at 120 °C for 8 h in a silicone oil bath. After the reaction completion, DMSO was removed on a rotary evaporator under reduced pressure. The resulting product was precipitated in THF, collected by filtration, purified by three successive precipitation using water as the solvent and methanol as the non-solvent, and dried under vacuum at ambient temperature for 24 h.

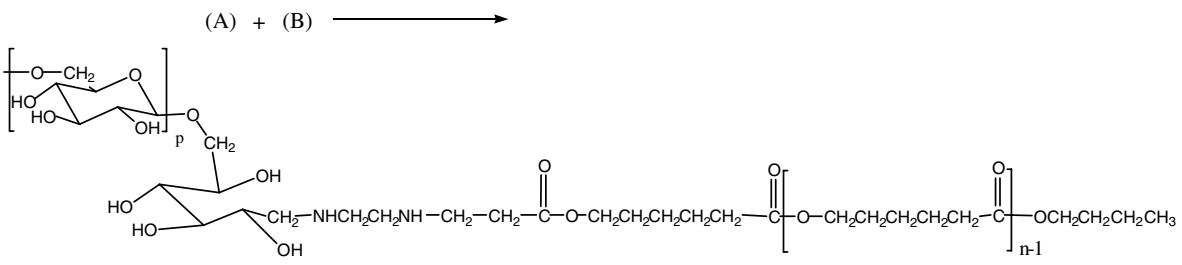
2.5. Measurements

The GPC measurement was performed at 40 °C, by using a Waters 515-410 gel permeation chromatograph equipped with Waters 410 detector and Ultrhydrogel column. Water was used as the eluent at a flow rate of 0.6 ml/min. ^{1}H NMR spectra were recorded on a Mercury-Plus 300(Varian, USA) spectrometer at 300 MHz, using tetramethysilane (TMS) as an internal standard and $CDCl_3$ or DMSO-d_6 as a solvent. The concentration of the solution is 5 mg/ml. The morphological examination of the copolymer micelles was performed using a JEM-2010HR high-resolution transmission electron microscope. A drop of PCL–Dextran block copolymer aqueous solution (2 mg/ml) containing 0.2 wt% phosphotungstic acid (PTA) was deposited onto a 200 mesh copper grid coated with carbon. Excessive solution was removed with a Kimwipes delicate wipe. The shape and size of the micelles were directly determined from each transmission electron micrograph. The micellar size and size distribution were determined by dynamic light scattering (DLS) using a BI-200SM Goniometer particle size analyzer (Brookhaven, USA). Each analysis lasted for 300 s and was performed at 23 °C with angle detection of 90°. The concentration of the polymer solution was 2 mg/ml. Steady-state fluorescence spectra were recorded on a Shimadzu RF-5301PC spectrofluorophotometer. Excitation spectra were monitored at 335 nm. The slit widths for both excitation and emission sides were maintained at 0.5 nm. Sample solutions were prepared by dissolving a predetermined amount of block copolymer in an aqueous pyrene solution of known concentration, and the solutions were allowed to stand for 48 h for equilibration.

3. Results and discussion

Fig. 1 gives the ^{1}H NMR spectrum of the poly(ε-caprolactone) end-capped with the acryloyl group. As seen, three peaks appeared at around 5.80–6.38 ppm could be attributed to the proton on the C=C double bond (Zhao, Zhang, Ma, Yang, & Yan, 2006). The signals at 0.91 ppm resulted from the proton of CH_3. All other absorption peaks were attributed to the protons of the poly(ε-caprolactone) backbone (Choi et al., 2006). Fig. 2 gives the ^{1}H NMR spectrum of the amino-functionalized dextran. The weak signal at 2.85 ppm is for the C-g proton of ethylene diamine adjacent to the methylene end group (Assumption & Mathias, 2003; Rannard & Davis, 2000; Rokicki & Piotrowska, 2002), and all other signals are for the proton of the dextran backbone (Shi & Zhang, 2006). Fig. 3 gives the ^{1}H NMR spectrum of the dextran–polycaprolactone block copolymer. The signals at 3.10 and 4.86 ppm are for the dextran (Shi & Zhang, 2006), and the signals at 1.28, 1.51, 2.22, 3.96 ppm are for the poly(ε-caprolactone) (Choi et al., 2006). This indicates that the Micheal reaction between the acryloyl group of Product **A** and the amino end group of Product **B** has occurred.

The microscopic characteristics of resultant amphiphilic block copolymer in aqueous medium were investigated using a fluorometer in the presence of pyrene as a fluorescent probe. It is known that the variation in the ratio (I_1/I_3) of intensity of first (372 nm) to the third (383 nm) vibronic peaks, the so-called polarity parameter, is quite sensitive to the polarity of microenvironment where the pyrene is located (Chen et al., 2006; Zhang, Lam, & Tan, 2005).



Scheme 3. Synthesis route of the dextran-*b*-PCL block copolymer.

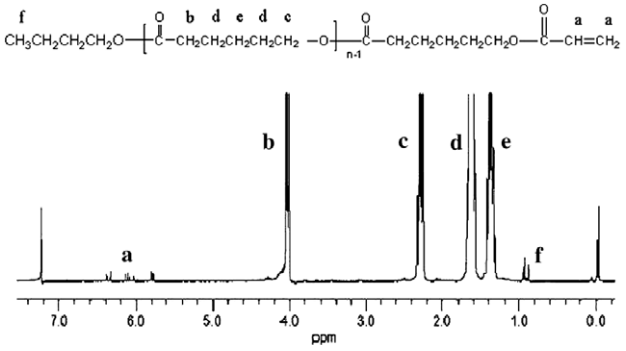


Fig. 1. ^{1}H NMR spectrum of allyl ended PCL ($CDCl_3$ as solvent).

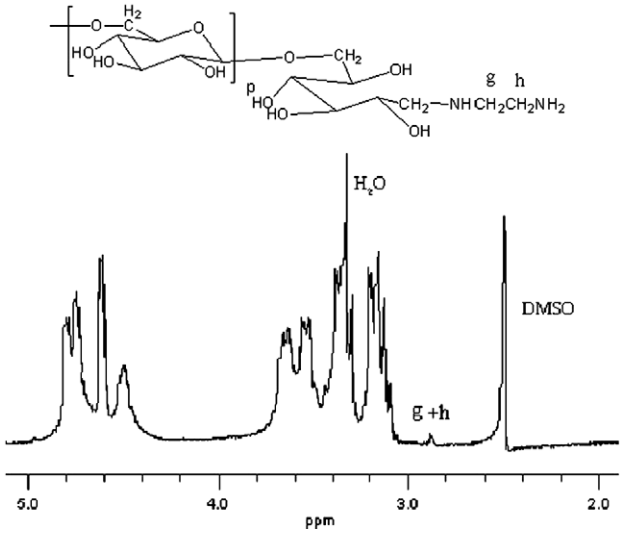


Fig. 2. ^{1}H NMR spectrum of the amino-functionalized dextran (DMSO-d^6 as solvent).

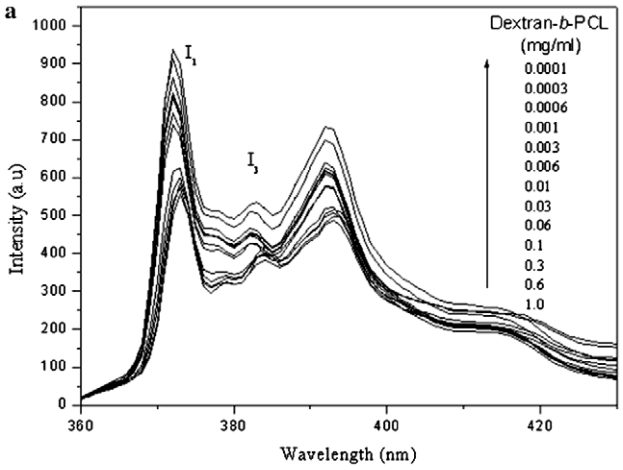


Fig. 4. (a) Fluorescence emission spectra of pyrene in water in the presence of the dextran-*b*-PCL block copolymer at 20 °C; (b) Change of the intensity ratio (I_1/I_3) versus the concentration of the dextran-*b*-PCL block copolymer at 20 °C.

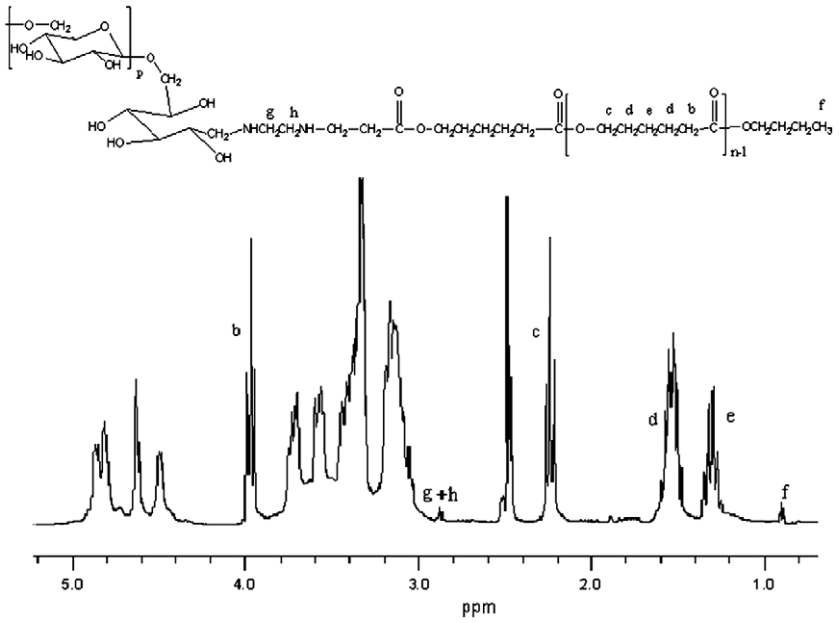


Fig. 3. ^{1}H NMR spectrum of the dextran-*b*-PCL block copolymer (DMSO-d^6 as solvent).

Fig. 4 gives the excitation spectra of pyrene in its aqueous solutions with various concentrations and the change of I_1/I_3 with the concentration. At lower concentrations, the I_1/I_3 values remain nearly unchanged. Further increasing the concentration, the intensity ratio starts to decrease, implying the micelle formation. The critical micelle concentration (*cmc*) was determined to be 0.06 mg/ml by the interception of two straight lines. Compared with low molecular weight surfactants (Zhang, 2001), the resultant amphiphilic block copolymer has a lower *cmc* value, indicating the stability of the micelles from this polysaccharide block copolymer at aqueous solution. Further work was carried out on the morphology of the formed micelles by the transmission electron microscopy (TEM) technique. From Fig. 5, it can be confirmed that the resulting polymeric micelles in water are spherical in shape, with the diameters ranged from 20 to 50 nm. The size distribution of the micelles was also investigated by the dynamic light scattering (DLS) technique. As shown in Fig. 6, a relative narrow unimodal distribution was obtained.

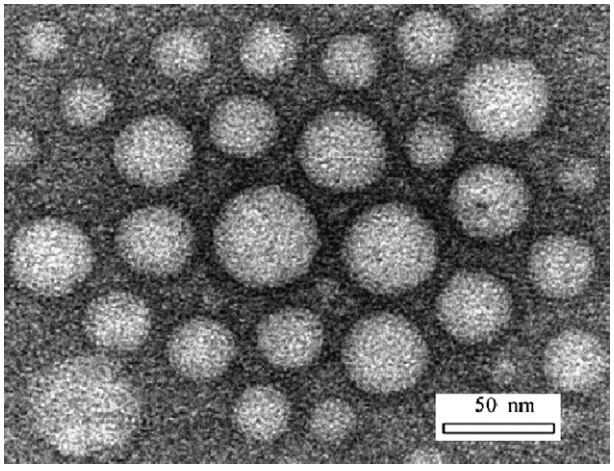


Fig. 5. Transmission electron microscopy photographs of the dextran-*b*-PCL block copolymer micelles.

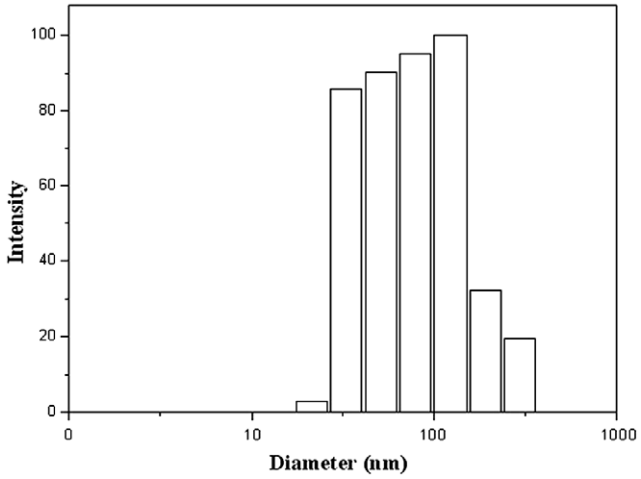


Fig. 6. The size distribution of the dextran-*b*-PCL block copolymer micelles in water.

In conclusion, a novel amphiphilic block copolymer was synthesized for the first time by the coupling reaction between the amino-functionalized dextran and the poly(ε-caprolactone) end-capped with the acryloyl group. It could self-assembly in water into polymeric micelles without any organic solvent or surfactant. Due to good hydrophilicity, biocompatibility, biodegradability and multifunctional conjugation capability of the used dextran, such polysaccharide derivative will hold greater advantages as the nanoscale container for hydrophobic drugs and genes when compared with widely used amphiphilic block copolymer of poly(ε-caprolactone) and polyethylene oxide or polyethylene glycol.

Acknowledgments

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