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Successful preferential formation of a novel macromolecular assembly—Trilayered polymeric micelle—That can incorporate hydrophilic compounds: The optimization of factors affecting the micelle formation from amphiphilic block copolymers

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ABSTRACT

We have developed a novel macromolecular assembly, trilayered polymeric micelle, which can incorporate hydrophilic compounds. The micelle can be prepared from the amphiphilic block copolymers without regard to their properties such as the copolymer's charges and the homogeneity of the copolymers forming the micelle's inner and outer parts. In this study, we investigated the optimal condition for the preferential formation of the trilayered polymeric micelle. GPC results clarified that the composition of the block copolymer, the concentration of PVA in the aqueous bulk phase, and the temperature during the preparation were the important preparation factors affecting preferential formation of the trilayered polymeric micelles. We successfully achieved the preferential formation of the trilayered polymeric micelles under optimal conditions. Furthermore, we confirmed that the model hydrophilic compound, FITC-dextran, was successfully encapsulated into the hydrophilic core of the trilayered polymeric micelles. The novel micelle that can incorporate hydrophilic compounds can have a variety of future medical applications such as a protein delivery-based therapy.

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1. Introduction

A gel is a three-dimensional polymer architecture that can retain a large amount of solvent. In particular, the hydrogel, a polymer network whose swelling medium is water, is a widely investigated biomaterial [1–5]. Since the flexible hydrogel can be formed from monomers and crosslinkers that can penetrate tissues, it has been said that the resulting hydrogel can fit the uneven surface of the tissues easily [4,6]. However, in general, hydrogels do not have the property of adhering to tissue surfaces strongly so that the fitted hydrogels gradually move from the site where they are placed. The acquisition of the tissue-adhesive property makes hydrogels attractive biomaterials for the possible development of indwelt or injectable devices for post-operative drug release. Furthermore, it has been widely reported that hydrophilic proteins can be loaded into hydrogels [1,7,8]. However, it is difficult to control the release properties of the proteins from hydrogels, because the release mechanism generally depends mainly on the phenomena that are difficult to be controlled, i.e., the degradation of the polymeric networks or the diffusion of the proteins through the medium of hydrogels.

On the other hand, the amphiphilic block copolymer can form a variety of nano-scaled self-assembled structures that can be available for medical use [9,10]. Several types of self-assembled structures, such as micelles, lamellar structures, and cylindrical structures, can be prepared from the amphiphilic block copolymers of which both the ratio and the property of both hydrophilic and hydrophobic blocks are rationally controlled [9]. It is well known that the polymeric micelle can both incorporate hydrophobic compounds into the hydrophobic core of the micelle and release drugs from the micelle by the dilution-induced collapse or the degradation of the micelle-forming polymers [10,11]. These properties have made the polymeric micelle an attractive material for the effective delivery of drugs to targeted sites in the body. Furthermore, we have developed a tissue-adhesive hydrogel covalently containing a reactive polymeric micelle as a crosslinker [12–14]. The hydrogel can release hydrophobic compounds in a controlled manner, because of both the inner core of the micelle acting as a compound container and the structure of the block copolymers

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regulating the compound-release properties. However, conventional polymeric micelles (i.e., bilayered polymeric micelles) cannot incorporate hydrophilic compounds. Consequently, the resulting hydrogel containing a reactive polymeric micelle as a crosslinker can incorporate and release only hydrophobic compounds, because of the hydrophobic environment of the inner core of the crosslinkable micelle.

From these points of view, we have developed a novel macromolecular assembly, trilayered polymeric micelles, which can incorporate hydrophilic compounds [15]. We prepared the trilayered polymeric micelles from the poly(ethylene glycol)-*b*-poly(lactic acid) (PEG–PLA) block copolymer which is widely used for the preparation of the biomaterials owing to its biocompatibility. The trilayered polymeric micelle can be prepared from the amphiphilic block copolymers without regard to their properties such as the copolymer's charges and the homogeneity of the copolymers forming the micelle's inner and outer parts. It is expected that the trilayered polymeric micelle that is incorporated into the hydrogels as a crosslinker can function as the container of the hydrophilic compounds. In particular, the resulting hydrogel can be a novel therapeutic hydrogel platform for releasing hydrophilic compounds like proteins in a sustained manner, because of the degradation of the block copolymer and the collapse of the micelle. However, the optimal conditions for the preparation of a trilayered polymeric micelle have not been clarified in detail.

This article clarifies the optimal conditions allowing for the preferential formation of the trilayered polymeric micelle. To obtain the optimal condition, we investigate the effects of several factors on the formation of the trilayered polymeric micelle, including the composition of the block copolymer, the volume of the aqueous bulk solution forming w/o emulsion, the concentration of the emulsion stabilizer in the aqueous bulk phase, the concentration of the block copolymer in the aqueous bulk phase, and the temperature during the emulsion preparation. Finally, we succeeded in preferentially preparing the trilayered polymeric micelle and reducing the formation of the by-product, bilayered polymeric micelle. The resulting micelle can be a novel attractive material for the efficient incorporation and controllable release of hydrophilic compounds.

2. Material and methods

2.1. Materials

We purified ethylene oxide (Sumitomo Seika Chemicals Co., Osaka, Japan) by distilling it in the presence of CaH_2. DL-Lactide (Tokyo Chemical Industry Co., Tokyo, Japan) was recrystallized twice from ethyl acetate. 3,3-Diethoxypropanol was distilled with sodium under reduced pressure. We synthesized potassium naphthalene by mixing potassium and naphthalene in anhydrous tetrahydrofuran (THF) for 18 h. Fluorescein isothiocyanate-dextran (FITC-dextran) with an average molecular weight of 20,000 and D_2O containing 0.75 w/w% 3-(trimethylsilyl)propionic-*2,2,3,3-d*$_4$ acid sodium salt were purchased from Sigma–Aldrich. Poly (vinyl alcohol) (PVA, the degree of polymerization: 500, saponification degree: 86–90 mol%) as an emulsion stabilizer was purchased from Wako Pure Chemical Industries and used without further purification. All other reagents were of analytical grade and were used without further purification.

2.2. Synthesis of acetal-terminated PEG–PLA block copolymers

Acetal-terminated PEG–PLA was synthesized by ring-opening polymerization of both ethylene oxide and DL-lactide in anhydrous THF according to the previously reported method with slight modifications [15]. 3,3-Diethoxypropanol (1.4–3 mmol) and potassium naphthalene (1.4–3 mmol) were mixed in THF for 1 h. The purified ethylene oxide (200–220 mmol) was then added to the obtained potassium 3,3-diethoxypropioxide solution (50 ml), and polymerization was carried out for 48 h at 25 °C. After polymerization, the purified DL-lactide (55–76 mmol) was added to the solution. The resulting polymer was precipitated into cold 2-propanol, stored in a freezer over night, centrifuged at 10,500 rpm for 15 min, and lyophilized in benzene. The number-average molecular weight of the block copolymer was determined by gel permeation chromatography (GPC) (column: TSKgel G3000H$_{HR}$, TOSOH, Japan, eluent: *N,N*-dimethylformamide containing 10 mM LiBr, flow: 1 ml/min, column temperature: 40 °C) and ^{1}H NMR (ECX-300, 300 MHz, JEOL Ltd., Tokyo).

2.3. Preparation of polymeric micelles

2.3.1. Preparation of the trilayered polymeric micelle (method 1)

A trilayered polymeric micelle was prepared according to the previously reported method with several modifications [15]. The mixed organic solvent (density was adjusted to 1.00 g/cm^3) was firstly prepared from 1,3-dimethyl-2-imidazolidinone (DMI) and *N,N*-dimethylacetoamide (DMA). A w/o emulsion was prepared by sonicating (15 min, in an ice bath) 2 ml of DMI-DMA mixed solvent containing PEG–PLA block copolymers (12.5 mg/ml) and 0.0625 ml of aqueous PVA solution (2 w/w%) containing FITC-dextran (10 mg/ml). Then, the w/o emulsion was added dropwise to an aqueous acetal-PEG–PLA block copolymer bulk solution. The volume of the aqueous bulk phase, the concentration of the block copolymer in the aqueous bulk phase, and the concentration of the PVA in the aqueous bulk phase was varied from 15 to 35 ml, from 2.85 to 17.1 mg/ml, and from 0 to 4 w/w%, respectively. The temperature of the aqueous acetal-PEG–PLA block copolymer solution was adjusted to be 4 °C or 25 °C. After 18 h of gentle stirring, the resulting solution containing trilayered micelles was dialyzed against water by means of a Spectra/Por7 dialysis membrane (molecular weight cut-off: 50 kDa; Spectrum, Houston, TX) for the removal of residual organic solvents and unincorporated FITC-dextran.

2.3.2. Preparation of the bilayered polymeric micelle (method 2)

A bilayered polymeric micelle was prepared according to the previously reported method with several modifications [12]. Acetal-PEG–PLA block copolymers were dissolved in 5 ml of DMA. Then, the solution of the acetal-PEG–PLA block copolymer was dialyzed against water by means of a dialysis membrane (molecular weight cut-off: 1 kDa; Spectrum, Houston, TX) for 24 h.

2.4. Characterization of the trilayered polymeric micelle

We performed GPC measurement to characterize the trilayered polymeric micelle (column: TSKgel G4000PW$_{XL}$, TOSOH, Japan, eluent: MilliQ water, flow: 1 ml/min, column temperature: 40 °C). To analyze the structure of the micelles, we performed ^{1}H NMR measurement by using ECX-300 (ECX-300, 300 MHz, JEOL Ltd., Tokyo). The micelles for the ^{1}H NMR measurement were prepared by means of their lyophilization after the dialysis procedure.

3. Results and discussion

3.1. Effects of the composition of the block copolymer

In general, the structure of the self-assembly formed from block copolymers is highly influenced by the composition or ratio of the hydrophilic and hydrophobic blocks of the block copolymer. A block copolymer with an unsuitable composition or ratio cannot form the most desirable self-assembled structure. Therefore, it is necessary to know the optimal composition or ratio of the block copolymer

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