A Novel Reactive Polymeric Micelle with Aldehyde Groups on Its Surface

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It is reported that amphiphilic AB block copolymers form in selective solvents micellar structures.[1] These nanospheric particles combine the advantages given by the hydrophobic core, which can act as a vehicle for medical drugs, and the unique properties of a hydrophilic shell.[2-4] PEO chains attached to a surface or forming the corona of a nanospheric particle in an aqueous medium exhibit rapid chain motions and have a large excluded volume. The steric repulsion resulting from a loss of configurational entropy of the bound PEO upon the approach of a foreign particle and the low interfacial free energy contribute to the extraordinary properties of PEO-covered surfaces.[5-10] The small size, apparent thermodynamic stability, and exceptional biological features of polymeric micelles favor their application in the biomedical field.[11-14]

Recently, a facile and quantitative synthetic method for the formation of heterobifunctional poly(ethylene oxide) was reported.[15,16] If one of the functional end groups in the heterobifunctional PEO can selectively initiate the polymerization of a hydrophobic monomer, a new heterobifunctional AB block copolymer can be created, retaining the other functional group (acetal group) at the PEO chain end. As a core-forming segment, polylactide (PLA) was chosen for the following reasons: (1) The ring-opening reaction of a lactide can be initiated by the living chain end of potassium ethylene oxide without any side reaction. (ii) PLAs are biodegradable and nontoxic polymers, which are widely utilized as implant materials. This is an important prerequisite considering a future application of this system in the biomedical field. (iii) Nanoparticles, consisting of block copolymers of α-methoxypoly(ethylene oxide) and PLA are suited for drug delivery.[17] Furthermore, the micelle formation and transformation of acetal into aldehyde groups on the micelle surface were discussed. An aldehyde group reacts rapidly with primary amino groups, forming a Schiff base, a chemical path which could be employed in the future for conjugations with proteins.

Experimental Section. Materials and Methods. Commercial tetrahydrofuran (THF), 3,3-diethoxypropanol, ethylene oxide (EO), and lactide were purified conventionally.[18] Potassium naphthalene was used as a THF solution.[19] GPC measurements were carried out using a Shimadzu 6A liquid chromatograph equipped with a PEO-calibrated TSK gel column (G4000HXL, G3000HXL, G2500HXL) and an internal RI detector (RID-6A). THF containing 2% of triethylamine was used as eluent at a flow rate of 1 mL/min. ^{1}H-NMR spectra were obtained using chloroform-*d* solutions with a JEOL EX400 spectrometer at 400 MHz.

Polymer Synthesis. α-Acetal-PEO-*b*-PLA was synthesized by a one-pot anionic ring-opening polymerization at room temperature under argon. One millimole (0.15 mL) of initiator (3,3-diethoxypropanol) and 1 mmol (3.5 mL) of potassium naphthalene were added to 20 mL of dry THF. After 10 min of stirring, 200 mmol (10 mL) of condensed EO was added via a cooled syringe to the formed potassium 3,3-diethoxypropoxide. The polymerization of EO proceeded for 2 days, resulting in a light brown, highly viscous solution. Additional potassium naphthalene (1.5 mL) was added until the reaction mixture turned green to stabilize the living chain end. Fifty millimoles (20 mL) of a lactide solution in THF (c = 2.5 mol/L) was introduced, and the polymerization proceeded for 90 min. The polymer was precipitated into a 30-fold excess of cold 2-propanol (−15 °C), stored for 2 h in the freezer, and centrifuged for 25 min at 4400 rpm. The polymer was freeze-dried in benzene, and 13.75 g of a white powder was obtained (yield: 86%).

Polymer Characterization. Molecular weights and polydispersities were determined by GPC: For PEO at the end of the EO polymerization, M_n = 5 700, M_w = 6 400 (130 EO units), and M_w/M_n = 1.12; at the end of the block copolymerization, M_n = 8 600, M_w = 10 300, and M_w/M_n = 1.20. The molecular weight for PLA was calculated from the difference: M_n = 2 900, M_w = 3 900 (20 LA units). The block copolymer structure was confirmed by ^{1}H-NMR spectroscopy.

Micelle Formation. The copolymer (200 mg) was dissolved in 40 mL of dimethyl acetamide, stirred at room temperature for 24 h, transferred into a preswollen membrane (Spectra/Por, molecular weight cutoff size 12 000–14 000), and dialyzed against water for 24 h. The water was exchanged after 2, 5, and 8 h. The aqueous micelle solution was frozen in liquid nitrogen and lyophilized. The yield of the micelle formation is 90%. To convert the acetal-terminated micelle into a micelle with aldehyde terminals, 50 mg of micelle was solubilized by sonication for 40 min in water and stirred for 3 h. The pH of the solution was then adjusted by HCl to pH 2, kept for 14 h, and readjusted to pH 7 by NaOH. The solution was lyophilized and dialyzed again against water for 24 h to remove the salt.

Micelle Characterization. A light scattering spectrophotometer (DLS-700, Photal, Otsuka Electronics) equipped with an Ar-ion laser was used at a wavelength of 488 nm to determine the micelle size. The scattering angles ranged from 30 to 150° and the concentration varied from 0.1 to 10 g/L. HPLC measurements were carried out using a JASCO 802-SC, 880-PU, 880-50, 851-AS chromatograph equipped with a Superose 6HR 10/30 column (Pharmacia Biotech) and an RI and UV detector (JASCO 830-RI, 870-UV) at a flow rate of 0.5 mL/min and a pressure of 5 kg/cm^2 with water as eluent. The micelle with terminal aldehyde groups was characterized after conversion with benzoic hydrazide by HPLC using an UV detector at 254 nm. A micelle solution (c = 1.0 g/L) was stirred with a 10-fold molar excess of the UV-active probe at room temperature for 30 min and then immediately analyzed by HPLC.

Results and Discussion. As reported previously[17,18] a potassium alkoxide initiator which possesses an acetal moiety can initiate the polymerization of EO without any side reaction to form heterotelechelic PEO having an acetal moiety at one end and a potassium alkoxide at the other end. This polymerization technique is also

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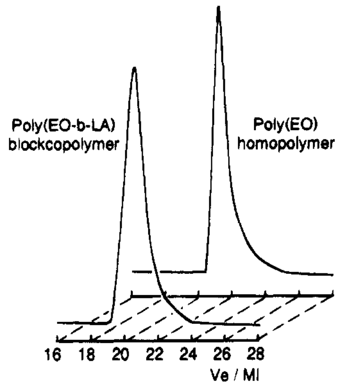


Figure 1. GPC chromatogram of poly(EO) homopolymer and poly(EO-*b*-LA) block copolymer.

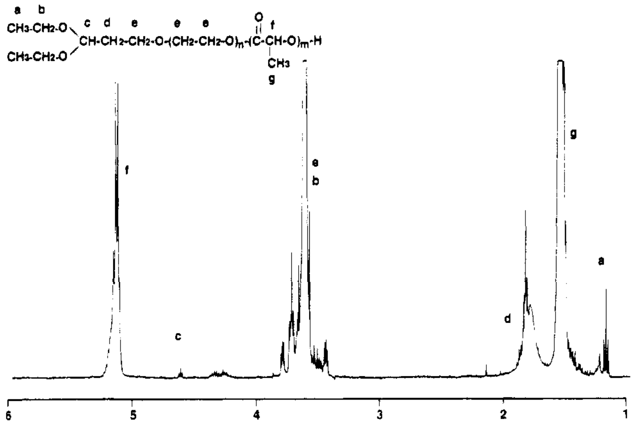
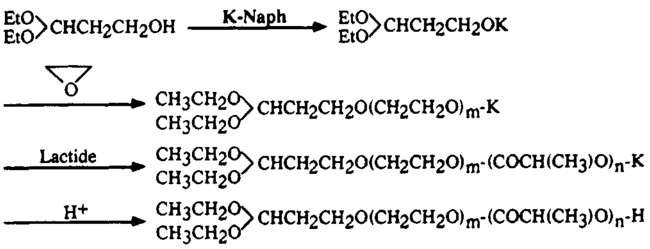


Figure 2. ^{1}H NMR spectrum of poly(EO-*b*-LA) with terminal acetal groups.

Scheme 1



applicable to a block copolymerization of EO with lactide, forming a block copolymer with an acetal group at the α-terminal in a one-pot synthesis. After the polymerization of EO with potassium 3,3-diethoxypropoxide as initiator, lactide was added to the reaction mixture. As shown in Figure 1, the molecular weight of the polymer increased, indicating effective block copolymer formation. The molecular weight of the block copolymer was controlled by the initiator to monomer ratio.

To obtain information about the functionality of the α-end, NMR analysis of the purified block copolymer was employed. Figure 2 shows the ^{1}H-NMR spectrum of the polymer. Referring to the literature on acetal-terminated PEO,[17] PLA[22] and diethoxypropyl alcohol as reference compounds, these signals were assigned and are described in the figure. Using the methyl proton of the acetal group at 1.2 ppm, the methylene proton of the PEO chain at 3.6 ppm, and the methine proton of the PLA chain at 5.2 ppm, the amount of acetal end groups was determined to be ca. 50%. By reducing the water content of the reaction mixture, the acetal content increased to ca. 90%, indicating that potassium 3,3-diethoxypropoxide was the sole initiating species (Scheme 1). For subsequent micelle studies a block copolymer containing 51.5% acetal end groups was used.

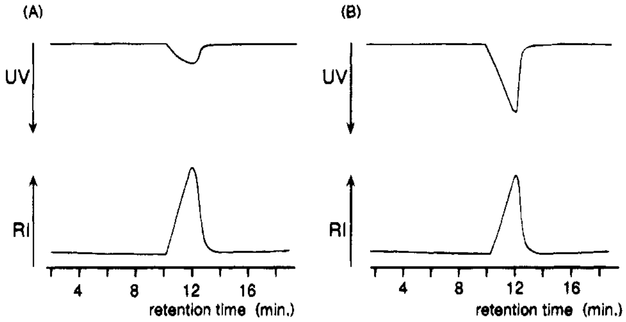


Figure 3. HPLC chromatograms of the CHO micelle before (left) and after (right) the reaction with benzoic hydrazide.

Dynamic light scattering of the solution provided information about the size and shape of the poly(EO-*b*-LA) micelle in water. The dependence of the micelle characteristics on the scattering angle and the sample concentration was investigated. Analyzing the data by the cumulant approach showed that the angular dependence of this system was sufficiently small to assume a spherical shape for the nanoparticle. Therefore, all further investigations were conducted at a scattering angle of 90°. Polydispersity, determined by the cumulant approach, ranged between 0.11 and 0.20, indicating a moderate polydispersity. The diffusion coefficient at indefinite dilution was determined to be 7.97×10^{-8} cm/s^2. By applying the Stokes–Einstein equation, a hydrodynamic radius of 29.3 nm was determined for this micelle at a scattering angle of 90°. This value was consistent with other data reported for micelle systems which are known to have a core–shell structure.[21,22]

The conversion of the acetal end group into an aldehyde end group was conducted after the micelle formation. The freeze-dried micelle was dissolved in water, and the solution was acidified by hydrochloric acid. The extent of conversion of acetal into aldehyde, determined by ^{1}H-NMR of the polymer, was ca. 22%, corresponding to a 40% conversion of the total acetal content. Under the given experimental conditions, an aldol condensation might have occurred to a certain extent and will be studied in more detail. It should be noted that no cleavage reaction of the PLA unit occurred, indicating the exclusion of the aqueous medium from the core of the micelle. After the transformation of the end groups, it was confirmed that the micelle diameter did not change.

To verify that surface aldehyde groups can be utilized for further modification reactions, a conjugation reaction with a model compound was conducted and monitored by HPLC. The micelle itself exhibits a weak UV signal due to the PLA core. As shown in Figure 3, the UV absorption of the micelles increased significantly after the reaction with benzoic hydrazide, retaining the elution at void volume, which indicated effective conjugation of the micelle with hydrazide, and keeping the micellar structure. The RI:UV ratio increased from 1:3.0 to 1:4.6 after the reaction between benzoic hydrazide and the aldehyde groups. No reaction occurred between acetal groups and benzoic hydrazide.

Conclusion. Heterobifunctional AB block copolymers consisting of PEO and PLA were synthesized containing an acetal terminal group at the PEO chain end. Evidence was presented which suggested that this block copolymer associated in water to form polymeric micelles. Acetal terminal groups located at the outer shell of the polymeric micelle were partially transformed into aldehyde groups after micellization. The degree of conversion was established by ^{1}H-NMR spectroscopy. Dynamic light scattering studies suggested a particle size of about 30 nm and a moderate polydispersity. The observed angular dependence was small enough to assume a spherical shape for this micelle.

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