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New biocompatible amphiphilic diblock copolymer based on dextran

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ABSTRACT

A new biocompatible amphiphilic diblock copolymer was obtained by end-to-end coupling of amino terminated dextran with an oligoester based on lithocholic acid. The bile acid oligoester was synthesized by polycondensation reaction of 3-succinoyloxy-derivative of lithocholic acid with diethylene glycol. The compounds were characterized by ^{1}H NMR and Fourier transform infrared spectroscopy. Size exclusion chromatography, thermal studies and wide-angle X-ray diffraction technique were used for oligoester and diblock copolymer characterization. The hydrodynamic diameter, size distribution and morphology of diblock copolymer self-aggregates formed in aqueous media were studied by dynamic light scattering and transmission electron microscopy. The critical aggregation concentration value for dextran copolymer (0.028 mg/mL) in aqueous solution was determined by fluorescence probe technique and it was lower than that of low molecular weight surfactants.

1. Introduction

Amphiphilic block copolymers, composed of hydrophilic and hydrophobic segments, and their self-assembly in aqueous solutions are presently a subject of great interest [1,2]. This attention is mostly motivated by their various applications as emulsifiers [3], surface coatings [1], drug delivery systems [4,5] or in nano-lithography [6]. Poly(ethylene glycol) was the polymer of choice as the hydrophilic segment of many amphiphilic block copolymers, but naturally occurring polysaccharides have recently gained attention as alternative hydrophilic polymers [7,8]. Among polysaccharides, dextran is widely used as blood-plasma substitute, drug carrier, or for protein stabilization, due to its biocompatibility, hydrophilicity, non-immunogenicity and nonfouling properties [9]. Moreover, dextran outer shells seem to provide a better colloidal stability to coated nanoparticles than PEG chains [10]. Up to now, a few dextran-based amphiphilic block copolymers were described, and the hydrophobic blocks were polystyrene [11–15], poly(ε-caprolactone) [16–19], poly(LD-lactide) [20,21], poly(alkyl-cyanoacrylate) [22,23], poly(γ-benzyl L-glutamate) [24] or poly(vinyl acetate) [25]. Most of the synthetic procedures take advantage of the presence of a single reactive reducing end in dextran, therefore the reductive amination of dextran

Abbreviations: DCC, dicyclohexylcarbodiimide; DCU, dicyclohexylurea; DEG, diethylene glycol; DMAP, 4 (N,N-dimethylamino)pyridine; EDA, 1,2-ethylenediamine; EEDQ, N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline; LCA, lithocholic acid; OES-LCA, oligoester of diethylene glycol and 3α-(succinoyloxy)-5β-cholan-24-oic acid; SC-LCA, 3α-(succinoyloxy)-5β-cholan-24-oic acid; TEA, triethylamine; *p*-TSA, *p*-toluen sulfonic acid.

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reductive end is the starting point for many block copolymer synthesis. The decisive role of this reaction is highlighted by the efforts to improve its yield and duration, for example by using microwave irradiation [26]. There are two main synthetic approaches applied for dextran block copolymers: (a) end-to-end coupling of the appropriately end functionalized preformed blocks, for example reductive amination of the dextran end groups by an amino semitelechelic polymer [11,14], reaction of end aminated dextran with a polymer with a carboxyl [19,20], acrylic [16] or aldehyde [17] end group, click reaction between blocks carrying propargyl and azide end groups [21,24], reducible disulfide bond formation between end thiol groups of the two blocks [18]; (b) preparation of a macroinitiator by a suitable sequence of chemical modifications at the dextran reducing end, followed by the growth of a synthetic polymer block by controlled radical polymerization [12,15,25], or redox radical emulsion polymerization [22,23]. End-to-end coupling is feasible for low molecular blocks and for synthetic polymers obtained by polycondensation (polyesters, polyamides). Use of dextran macroinitiators affords block copolymers with high molar masses, is applicable mainly for growing synthetic polymer blocks by radical or ring opening polymerization, and often requires dextran's OH group protection. Size and morphology (micelles or vesicles) of the formed aggregates were mainly influenced by the relative length of the two blocks [13,19,24]. Application envisaged for these new self-assembling natural-synthetic polymers included surface active agents [11], emulsifying agents [22,25], drug delivery systems [17,18,20,21] or honeycomb structured porous films [15].

In a previous paper we described the synthesis of amphiphilic block-like polymers by dextran end modification with a hydrophobic group, alkyl or a bile acid, and found that these end modified dextrans can form micelles in water [27]. In the present article, we report the synthesis of a novel amphiphilic block copolymer by end-modification of dextran with a longer hydrophobic chain containing a bile acid, namely an oligoester of the lithocholic acid, which could lead to a polymer with improved self-assembling properties. In order to obtain such a material, the oligoester of 3-succinoyloxy-lithocholic acid with diethylene glycol (DEG) and the amino-functionalized dextran were prepared and their subsequent coupling reaction was achieved. Crystallinity and thermal stability were analyzed for the lithocholic acid oligoester as well as for the dextran diblock copolymer. Diblock copolymer capacity to form micelles in aqueous solution and micelle properties was also investigated. To the best of our knowledge, this is the first report on the biocompatible block copolymers based on a polysaccharide (semi-flexible hydrophilic segment) and a bile acid oligoester (rigid hydrophobic segment).

2. Materials and methods

2.1. Materials

Dextran from Leuconostoc mesenteroides (M_r = 6000 Da, as reported by supplier) was bought from Sigma. Its weight-average (M_w) (6385 Da) and number-average molar masses (M_n) (4500 Da) were determined by size exclusion chromatography, using dextran standards for calibration [27]. Diethylene glycol (DEG) and lithocholic acid (LCA) were purchased also from Sigma. Sodium borohydride ($NaBH_4$) was bought from Fluka. *p*-Toluen sulfonic acid (*p*-TSA), purchased from Aldrich, was recrystallized twice from chloroform. All the other reagents of analytical grade were from Aldrich and were purified/dried by standard methods. Dialysis tubing (MWCO 12,000) was purchased from Sigma–Aldrich.

2.2. Synthetic procedures

2.2.1. Synthesis of 3α-(succinoyloxy)-5β-cholan-24-oic acid (SC-LCA)

The lithocholic acid derivative was synthesized by a procedure described in detail elsewhere [28–30]. Briefly, LCA (10 g, 0.0266 mol) and succinic anhydride (2.91 g, 0.029 mol) were dissolved in 100 mL dry $CHCl_3$. Triethylamine (TEA) (4.07 mL, 0.0292 mol) was then added and the mixture was stirred under reflux for 24 h. The solvent was removed by distillation and the residue was dispersed in 500 mL distilled water, the pH of which was adjusted to 3 with concentrated HCl. The solid recovered by filtration was further purified by precipitation of a dimethylsulfoxide (DMSO) solution in water, and then dried under vacuum at ambient temperature for 24 h. A white solid (9.98 g, 82% yield) with melting point 228.6 °C was obtained. FT-IR (cm^{-1}): 1707.08 ($\nu_{C=O\text{-}acid}$), 1724.68 ($\nu_{C=O\text{-}ester}$) (Fig. 1S); ^1H NMR (400 MHz, DMSO-d_6), δ (ppm) = 0.68 (s, 3H, $C^{18}H_3$); 0.92 (s, 3H, $C^{19}H_3$); 0.98 (3H, d, $C^{21}H_3$); 1–2 (m, 24H, steroidal protons); 2.23–2.34 (dd, 2H, $C^{23}H_2$); 2.59–2.64 (dd, 4H, OC—$C^{26}H_2$—$C^{27}H_2$—CO); 4.65 (m, 1H, C^3H—O—CO). Elemental analysis for $C_{28}H_{44}O_6$, calculated (found)(wt%): C 71.03 (70.6); H 10.04 (9.24).

2.2.2. Synthesis of oligoester of DEG and 3α-(succinoyloxy)-5β-cholan-24-oic acid (OES-LCA)

SC-LCA (5 g, 0.011 mol), DEG (1.1 mL, 0.011 mol) and an equimolar mixture of 4-N,N-dimethylaminopyridine (DMAP) (0.3 g, 0.0025 mol) and p-toluenesulfonic acid (*p*-TSA) (0.43 g, 0.0025 mol) were dissolved in 50 mL dichloromethane (DCM) and 5 mL pyridine. Dicyclohexylcarbodiimide (DCC) (5.78 g, 0.028 mol), dissolved in 50 mL DCM, was added and the mixture was stirred at room temperature for 48 h. The mixture was filtered for removing the precipitated dicyclohexylurea (DCU) and the filtrate was precipitated into water. After filtration, the precipitate was washed on the filter with distilled water, the pH of which was adjusted to 3 with concentrated HCl. The precipitate was washed on the filter with distilled water, then dried under vacuum at ambient temperature for 24 h. A white solid (5.2 g, 87% yield) was obtained. FT-IR (cm^{-1}): 1734.87 ($\nu_{C=O\ ester}$ and $\nu_{C=O\text{-}acid}$) (Fig. 2S); ^1H NMR (400 MHz, $CDCl_3$), δ (ppm) = 0.61 (s, 3H, $C^{18}H_3$); 0.81