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Self-assembled cellulose particles for agrochemical applications

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ABSTRACT

The present work focuses on the hydrophobic functionalization of water soluble celluloses methyl cellulose, hydroxyethyl cellulose and (hydroxypropyl)methyl cellulose with the anticancer steroid diosgenin, and two synthetic brassinosteroids (DI31 and S7) used as agrochemicals. Fourier transform infrared (FT-IR) and nuclear magnetic resonance (NMR) spectroscopies confirmed the cellulose modification. Prepared amphiphilic steroid-cellulose conjugates can self-assemble in water as stable and almost neutral particles with micelle-like structure, as depicted using dynamic light scattering. Whereas scanning and transmission electron microscopies showed 50–300 nm almost spherical particles and aggregates in dried state, atomic force microscopy assessed particles aggregates with mean sizes of 220–355 nm. These cellulose particles showed sustained steroid release in acidic aqueous medium over 72 h, and good stimulatory agrochemical activity in radish cotyledons assay. Thus, the outlined synthesis of steroid-cellulose conjugates, which would be capable to form self-assembled particles in water for controlled release of agrochemicals, is envisioned as a promising strategy.

1. Introduction

Cellulose, a natural linear polysaccharide based on repeating units of β(1 → 4) linked D-glucose, is the most abundant biopolymer as the key structural component of plants (33% of vegetal material). It combines biocompatibility, good biodegradability (glycoside hydrolases and cellulase enzymes in some ruminants, termites and fungi) and no toxicity, while exhibiting proper reactivity towards esterification [1–3]. Even, when cellulose itself is not soluble in water, several cellulose esters show proper aqueous solubility, or are able to form stable aqueous nanoparticulate or micelle dispersions after further functionalization [4,5]. In this sense, methyl cellulose (MC), hydroxyethyl cellulose (HEC) and (hydroxypropyl)methyl cellulose (HPMC) are water-soluble cellulose esters widely used in food and pharmaceutical industry, and envisioned as promising materials for novel smart medicines [6–8]. Particularly, HPMC based drug delivery systems are well established in medical applications due to the polymer matrix biocompatibility and swelling properties upon contact with biological fluids [9–11]. Thus, cellulose based micro/nanoparticles, hydrogels, fibers, films and composites have been proposed for different medical applications (e.g. antibiotic and anticancer drug delivery) [12–16]. An added value of cellulose-based systems is their good biodegradability [17,18], antimicrobial behaviour observed after cationization or when prepared as composites or other formulations against *L. monocytogenes* and *E. coli* [14], and low cytotoxicity [15,19]. On the other hand, the self-assembly of stimuli-responsive amphiphilic celluloses as nanoparticulate systems for sustained release of different drugs is an active

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research area [5,20]. However, these self-assembled systems are mostly devoted to release of loaded hydrophobic drugs [5,20], instead of delivery of the covalently grafted compound. Furthermore, reports about preparation of agrochemical controlled delivery systems are not as common as the ones devoted to medicine. Our research extends previous work on the field of synthesis and assay of different polymer-based systems for sustained release of brassinosteroids for agriculture [21].

Diosgenin ((25R)-spirost-5-en-3β-ol) is a steroidal sapogenin mostly obtained by basic hydrolysis of dioscin, the most available steroidal saponin. Both, dioscin and the derived diosgenin, exhibit antioxidant, anti-inflammatory, estrogenic activity, and cytotoxicity to some cancer cell lines [22–24]. Diosgenin is the main substrate in chemical synthesis of some steroids (i.e. progesterone, corticosteroids, and contraceptives), due to the fact that the required backbone and stereochemistry are already present in diosgenin [24]. In this sense, diosgenin is the precursor of two Cuban synthetic analogues of brassinosteroids (DI31 and S7) [25] used as commercial agrochemicals over the last two decades (Biobras-16). Biobras-16 regulates plants growth and protects the crops of biotic and abiotic stress once applied, with increases in harvest of 5–25% [26,27]. Nevertheless, the expected agrochemical benefits are not fully achieved in plants because the exogenous brassinosteroids are rapidly metabolized. Consequently, up to two or three foliar spray applications are usually applied to crops, which increase economic cost of the Biobras-16 application [26]. Moreover, the hydrophobicity of brassinosteroids DI31 and S7 limits their bioavailability to plants and commercial Biobras-16 formulation includes plenty of ethanol, and some environmentally unfriendly additives (i.e. N,N-dimethylformamide, surfactants). Herein, it is proposed that synthesis of novel biodegradable conjugates of diosgenin, DI31 and S7, by conjugation to water soluble cellulose esters via hydrolysable ester bonds, should improve bioavailability of the parent steroids and provide their sustained release over time. In the present research, we synthesised steroid-cellulose conjugates functionalized with three different steroids linked via ester bond, characterized them by attenuated total reflectance Fourier transform infrared (ATR-FTIR), proton nuclear magnetic resonance (^{1}H NMR) and bi-dimensional nuclear magnetic resonance (2D-NMR) spectroscopies, as well as assessed self-assembly of these conjugates by dynamic light scattering, atomic force microscopy, scanning and transmission electron microscopies. *In vitro* drug release of the steroids from conjugates was investigated in an acidic aqueous medium. *In vitro* agrochemical activity of the prepared cellulose nanoparticles towards radish (*Raphanus sativus*) was also studied. To the best of our knowledge, this is the first approach to the preparation of cellulose self-assembled particulate-based system for the delivery of brassinosteroids as agrochemicals.

2. Experimental

2.1. Materials

Three water soluble celluloses named methyl cellulose (MC) (14 mPa s 2% in water at 20 °C, methoxyl content 30.2%, number-average molecular weight Mn ca. 14,000 g/mol), hydroxyethyl cellulose (HEC) (178.6 mPa s 1% in water at 20 °C, Mn ca. 220,000 g/mol) or (hydroxypropyl)methyl cellulose (HPMC) (22.1 mPa s 2% in water at 25 °C, methoxyl content 28.8% and hydroxypropyl content 8.9%, Mn ca. 25,000 g/mol) (Sigma A.G.) were used to prepare the steroid-cellulose conjugates. Solvents and chemicals were employed as purchased from Sigma-Aldrich. The diosgenin and synthetic analogues of brassinosteroids (DI31 and S7) were supplied by the Center of Natural Products at University of Havana, Cuba. Hemisuccinates of diosgenin and two synthetic analogues of brassinosteroids with agrochemical activity (DI-31 and S7) were synthesised by base-catalyzed traditional esterification in pyridine with succinic anhydride [28].

2.2. Synthesis of steroid-cellulose conjugates

100 mg (0.4–0.6 mmol monosaccharide units) of methyl cellulose (MC), hydroxyethyl cellulose (HEC) or (hydroxypropyl)methyl cellulose (HPMC) were stirred 48 h at room temperature with 20 mg (0.05 mmol) of diosgenin or two synthetic brassinosteroid DI31 and S7 hemisuccinates (MSD, MSDI31 and MSS7), with 20 mg (0.1 mmol) of 1-ethyl-3-(3′-dimethylamino)carbodiimide hydrochloride and 20 mg (0.16 mmol) of 4-(dimethylamino)pyridine in 10% LiCl in N,N-dimethylacetamide. Products were dialyzed (Spectra/Por 6, MWCO 1 kDa, Spectrum Lab., USA) against methanol (1 time, 600 mL, 12 h) and bi-distilled water (2 times, 1 L, 24 h), and lyophilized affording white cotton wool like products. Dissolution of studied celluloses in 10% LiCl in N,N-dimethylacetamide prior to chemical reaction was conducted [29]. All studies were performed in triplicate for each sample.

2.3. Preparation of the self-assembled particles

The synthesised steroid-cellulose conjugates were able to form nanoparticles in aqueous solution after stirring overnight. To this end, the cellulose conjugates (ca. 0.5–2.0 mg/mL) were stirred overnight at 100 rpm in bi-distilled water or phosphate buffered saline solution (PBS, pH 7.4).

2.4. Characterization

The number-average molecular weight of celluloses and steroid-cellulose conjugates were determined with gel permeation chromatography (GPC) using a Viscotek GPCmax (Malvern, Germany) with a PFG column from PSS, 300 × 8 mm^2, 5 µm particle size. The samples (100 µL of injection volume, 2 mg/mL) were eluted with 0.01 mol/L LiBr in (N,N)-dimethylformamide at a flow rate of 0.75 mL/min at 60 °C. The cellulose solutions were filtered through a 0.22 µm microporous nylon film syringe filter (Macherey-Nagel, Germany). The molecular weights were determined with a Viscotek TDA 305 Triple Detector Array (Malvern,