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Environmentally friendly pathways towards the synthesis of vinyl-based oligocelluloses

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ABSTRACT

The synthesis of vinyl-based oligocelluloses using cellodextrin phosphorylase as biocatalyst in buffer solution is presented. Various types of vinyl glucosides bearing (meth)acrylates/(meth)acrylamides functionalities served as the glucosyl acceptor in the enzyme catalyzed reverse phosphorolysis reaction and α-glucose 1-phosphate as the glucosyl donor. The enzymatic reaction was followed by thin layer chromatography and the isolated product yields were about 65%. The synthesized vinyl-based oligocelluloses had an average number of repeating glucosyl units and a number average molecular weight up to 8.9 and 1553 g mol^{-1}, respectively. The majority of the bonds at the alpha position of acrylate units in oligocellulosyl-ethyl acrylate was fragmented as characterized by ^{1}H NMR spectroscopy and MALDI-ToF spectrometry. Nevertheless, a minor amount of fragmentation was observed in oligocellulosyl-ethyl methacrylate and oligocellulosyl-butyl acrylate but no fragmentation was detected in the (meth)acrylamide-based oligocelluloses. Crystal lattice of the prepared vinyl-based oligocelluloses was investigated *via* wide-angle X-ray diffraction experiments.

1. Introduction

Cellulose is the most abundant biopolymer on earth and has been widely used in our daily lives mainly for paper products, composites, and building materials (Huber et al., 2012; Klemm, Schmauder, & Heinze, 2002; Moon, Martini, Nairn, Simonsen, & Youngblood, 2011; Nakajima, Dijkstra, & Loos, 2017; Yates, Ferguson, Binns, & Hartless, 2013). Cellulose is a linear polymer which consists of a hundred to a thousand glucosyl units linked through β-(1 → 4)-glycosidic bonds. Cellulose oligomers or cellooligosaccharides, later mentioned as oligocelluloses, typically contain only a few glucosyl units and gained some interest in the last decades especially because of their properties which are essentially the same as natural cellulose. Besides, these materials have potential applications for non-digestible dietary fiber products (Mussatto & Mancilha, 2007; Satouchi et al., 1996; Watanabe, 1998; Yamasaki, Ibuki, Yaginuma, & Tamura, 2008), novel bio-based surfactants (Billès, Onwukamike, Coma, Grelier, & Peruch, 2016; Hato, Minamikawa, Tamada, Baba, & Tanabe, 1999; Kamitakahara, Nakatsubo, & Klemm, 2007), hybrid nanomaterials (Enomoto-Rogers, Kamitakahara, Yoshinaga, & Takano, 2010, 2011b), and scaffold candidates for tissue engineering (Wang, Niu, Sawada, Shao, & Serizawa, 2017).

In general, two methods have been utilized to obtain oligocelluloses: (1) Degradation of natural cellulose and (2) synthetic pathways *via* chemical or enzymatic reactions (Billès, Coma, Peruch, & Grelier, 2017). The first method is easy to be performed since it just requires relatively cheap acidic reagents, however, this route has less control over the chemical and crystalline structures of the products. In addition, not only oligocelluloses but also unwanted furanic by-products will be formed rendering fractionation/purification steps of the reaction mixture necessary. The chemical synthesis is based on ring-opening polymerization of structurally-modified glucopyranoses (Nakatsubo, Kamitakahara, & Hori, 1996; Xiao & Grinstaff, 2017) and glucosylation reactions between glucosyl donors and glucosyl acceptors (Kamitakahara, Nakatsubo, & Klemm, 2006; Kamitakahara et al., 2007). Even though well-defined oligomers with high purity can be achieved, these approaches are time-consuming due to multi-step reactions involved in the precursor's synthesis.

In vitro enzymatic synthesis of oligocelluloses provides some advantages compared to the previous methods; for example, well-controlled structures of products are obtained in a one-step polymerization owing to high regio-, *enantio*-, chemo-, and stereoselectivities of the enzymes. Moreover, enzymes are non-toxic compounds, isolated from sustainable resources, and catalyze the reaction under mild environments (Fodor, Golkaram, van Dijken, Woortman, & Loos, 2017; Loos, 2010; Palmans & Heise, 2011; Shoda, Uyama, Kadokawa, Kimura, &

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Kobayashi, 2016).

Cellulases (Egusa, Kitaoka, Goto, & Wariishi, 2007; Fort et al., 2000; Kobayashi, Kashiwa, Kawasaki, & Shoda, 1991) and cellodextrin phosphorylases (CdP's) (Nakai, Kitaoka, Svensson, & Ohtsubo, 2013; O'Neill & Field, 2015; Puchart, 2015) are the most exploited enzymes for the production of synthetic oligocelluloses. Cellulases can catalyze the polycondensation reaction of β-cellobiosyl fluorides and the reaction is necessarily performed in organic solvent/buffer mixtures to maintain the products solubility and to prevent the products hydrolysis – facilitated by the enzyme itself. On the other hand, CdP's are able to accept a broader range of substrates such as glucose (Hiraishi et al., 2009; Serizawa, Kato, Okura, Sawada, & Wada, 2016), cellobiose (Nakai et al., 2010; Petrović, Kok, Woortman, Ćirić, & Loos, 2015), and various cellodextrins (Sawano, Saburi, Hamura, Matsui, & Mori, 2013) for the synthesis of oligocelluloses *via* a reverse phosphorolysis mechanism in aqueous media. The effort to apply unnatural substrates for CdP from *Clostridium thermocellum* (CtCdP) was first studied by Serizawa and coworkers (Nohara, Sawada, Tanaka, & Serizawa, 2016, 2017; Wang et al., 2017; Yataka, Sawada, & Serizawa, 2015, 2016). They utilized monofunctional glucose, in which the anomeric carbon was chemically bonded either with alkyl, amine, azide, oligo(ethylene glycol) or methacrylate groups in order to provide additional reactivities of the prepared oligocelluloses with other molecules or to control their self-assembly processes.

In this report, we extend the range of structures reported and present different novel types of vinyl glucosides – glucosyl-ethyl acrylate, glucosyl-ethyl methacrylate, glucosyl-butyl acrylate, glucosyl-ethyl acrylamide, and glucosyl-ethyl methacrylamide – as promising substrates for CtCdP in the synthesis of vinyl-based oligocelluloses whereby α-glucose 1-phosphate served as the glucosyl donor. The used vinyl glucosides, that were uniquely characterized to be anomerically pure and monofunctional, were synthesized enzymatically under environmentally benign conditions (Adharis, Vesper, Koning, & Loos, 2018; Kloosterman, Roest, Priatna, Stavila, & Loos, 2014). In addition, the hydroxyalkyl (meth)acrylates/(meth)acrylamides, the source of the vinyl groups, can be synthesized using bio-based precursors of acrylic acid (Beerthuis, Rothenberg, & Shiju, 2015), methacrylic acid (Lansing, Murray, & Moser, 2017), and ethylene glycols (Beine, Hausoul, & Palkovits, 2016). Hence, the overall reaction can be considered as a green route towards the production of vinyl-based oligocelluloses, which is due to the choice of starting materials, catalysts, and solvent. The starting materials were derived from renewable feedstocks, whereas enzymes were used as the biocatalyst and the utilized solvent was a water based buffer solution. Furthermore, vinyl groups available at the reducing end of the oligocelluloses offer high reactivity and versatility for further (co)polymerization with different monomers, resulting in polymers with novel physical and chemical properties. For instance, the synthesized (co)polymers can be applied as promising bio-based materials like hydrogels (De France, Hoare, & Cranston, 2017; Hata et al., 2017; Wang et al., 2017), polymeric surfactants (Cao & Li, 2002; Enomoto-Rogers, Kamitakahara, Yoshinaga, & Takano, 2011a), compatibilizer (Yagi, Kasuya, & Fukuda, 2010), and as well-defined nanostructure materials (Kamitakahara, Baba, Yoshinaga, Suhara, & Takano, 2014; Otsuka et al., 2012; Sakaguchi, Ohura, & Iwata, 2012). The synthesized vinyl-based oligocelluloses were successfully characterized by proton nuclear magnetic resonance spectroscopy, size exclusion chromatography, wide-angle X-ray diffraction, and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

2. Experimental

An experimental roadmap for the synthesis and characterization of the vinyl-based oligocelluloses is presented in Fig. 1. The materials used for the synthesis, the characterization methods, as well as the synthesis procedures are outlined in the following paragraphs.

2.1. Materials

α-D-Glucose 1-phosphate disodium salt hydrate ≥97% (α-Glc1*P*) and *n*-butanol (*n*-BuOH) were purchased from Sigma-Aldrich. Cellobiose 98% was purchased from Acros Organics. Ethanol (EtOH), isopropyl alcohol (IPA), and concentrated H_2SO_4 were acquired from Avantor. Unless otherwise mentioned, all chemicals were used as received. Five types of vinyl glucosides consist of glucosyl-ethyl acrylate (G-EA), glucosyl − ethyl methacrylate (G-EMA), glucosyl − butyl acrylate (G-BA), glucosyl − ethyl acrylamide (G-EAAm), and glucosyl − ethyl methacrylamide (G-EMAAm) were synthesized according to the literature (Adharis et al., 2018; Kloosterman et al., 2014). CtCdP was expressed in *Escherichia coli* BL21-Gold-(DE3) strain harboring pET28a-CtCdP plasmid and purified as reported before (Petrović et al., 2015). The activity of the enzyme was 15.2 units per ml of stock solution, equal to 0.13 units per ml of the reaction mixture (One unit was defined as the amount of enzyme that converts 1 μmol of substrate per minute under HEPES buffer pH 7.5 at 45 °C).

2.2. Methods

2.2.1. Thin layer chromatography (TLC)

TLC was carried out on aluminum sheet silica gel 60/kieselguhr (Merck) using eluent of *n*-BuOH/IPA/H_2O (1/2.5/1.5). Spot visualization of the products was performed by spraying the TLC plate with 5% H_2SO_4 in EtOH followed by heating.

2.2.2. ^{1}H nuclear magnetic resonance (NMR) spectroscopy

^{1}H NMR spectra were recorded on a 400 MHz Varian VXR Spectrometer using 4 wt% sodium deuteroxide (Aldrich) in deuterium oxide (99.9 atom% D, Aldrich) as the solvent. The acquired spectra were processed by MestReNova Software from Mestrelab Research S.L.

The average degree of polymerization (DP_n) of the vinyl-based oligocelluloses was calculated from the ^{1}H NMR spectra (Fig. 3) using Eq. (1) while DP_n of the native oligocellulose was determined using Eq. (2). $H1$, $H2$, and $H11_{trans}$ represent the peak integration of anomeric proton on C1 position, proton on C2 position, and one of the protons of the vinyl groups in the vinyl-based oligocelluloses, respectively. Furthermore, $H\alpha$ and $H\beta$ are equal to the peak integration of alpha-anomeric and beta-anomeric protons of the native oligocellulose.

$$DP_n = \frac{H1 + H2}{H11_{trans}} \quad (1)$$

$$DP_n = \frac{H\alpha + H\beta + H2}{H\alpha + H\beta} \quad (2)$$

The number-average molecular weights (M_n) of the vinyl-based and native oligocelluloses were determined *via* Eq. (3) where M_o and B are the molecular weights of dehydrated glucose and hydroxy-alkyl (meth) acrylate/(meth)acrylamide units (or water molecule), respectively.

$$M_n = (DP_n \times M_o) + B \quad (3)$$

2.2.3. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF MS)

MALDI-ToF MS was executed on a Voyager DE-PRO instrument from Applied Biosystems in the positive and linear mode. In a MALDI-ToF MS plate, 0.5 μl of oligocellulose suspensions (2–5 mg/ml) was mixed with 1.0 μl of matrix solution (10 mg of 2,5-dihydroxybenzoic acid in 1 ml of 50 v% H_2O, 50 v% acetonitrile, 0.01 v% trifluoroacetic acid). The obtained spectra were analyzed using Data Explorer Software from Applied Biosystems.

Weight-average molecular weight (M_w), M_n, and polydispersity index (PDI) of the vinyl-based and native oligocelluloses were determined from the MALDI-ToF spectra (Fig. 4) by Eqs. (4)–(6), respectively, where N_i and M_i refer to the area below the peak and the molar