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Environmental 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 \((\beta (1 \rightarrow 4)\)-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 glycopyranoses (Nakatsubo, Kamitakahara, & Hori, 1996; Xiao & Grinstaff, 2017) and glycosylation 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, & Nakatsubo, 2017).

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Kobayashi, 2016).

Cellulases (Fugusa, Kitaoka, Goto, & Wariishi, 2007; Fort et al., 2000; Kobayashi, Kashiwa, Kawasaki, & Shoda, 1991) and cellobextrin 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 (Hiraiishi et al., 2009; Serizawa, Kato, Okura, Sawada, & Wada, 2016), cellobiose (Nakai et al., 2010; Petrović, Kok, Woortman, Ćirić, & Loos, 2015), and various cellobextrins (Sawano, Saburi, Hamura, Matsui, & Morī, 2013) for the synthesis of oligocelluloses via a reverse phosphorylization mechanism in aqueous media. The effort to apply unnatural substrates for CdP from Clostridium thermocellum (CrCdP) 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 glycosyl donor. The used vinyl glucosides, that were uniquely characterized to be anomerically pure and monofunctional, were synthesized enzymatically under environment-friendly benign conditions (Adharis, Vesper, Koning, & Loos, 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. 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/H2O (1/2.5/1.5). Spot visualization of the products was performed by spraying the TLC plate with 5% H2SO4 in EtOH followed by heating.

2.2.2. 1H nuclear magnetic resonance (NMR) spectroscopy

1H NMR spectra were recorded on a 400 MHz Varian VXR Spectrometer using 4 wt% sodium deuterium oxide (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 (DPn) of the vinyl-based oligocelluloses was calculated from the 1H NMR spectra (Fig. 3) using Eq. (1) while DPp of the native oligocellulose was determined using Eq. (2). H1, H2, and H1trans represent the peak integration of anomic proton on C1 position, proton on C2 position, and one of the protons of the vinyl groups in the vinyl-based oligocelluloses, respectively. Furthermore, Ha and Hb are equal to the peak integration of alpha-anomeric and beta-anomeric protons of the native oligocellulose.

\[
\text{DPn} = \frac{H1 + H2}{H1\text{trans}}
\]

\[
\text{DPp} = \frac{Ha + Hb + H2}{Ha + Hb}
\]

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

\[
M_n = (DP_n \times M_o) + B
\]

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% H2O, 50 v% acetonitrile, 0.01 v% trifluoroacetic acid). The obtained spectra were analyzed using Data Explorer Software from Applied Biosystems.

Weight-average molecular weight (Mw), Mw, 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 N1 and M1 refer to the area below the peak and the molar...
2.2.5. Wide-angle X-ray diffraction (WAXD)

WAXD was carried out using a Bruker Advance diffractometer (Cu Kα radiation, λ = 0.1542 nm) in the angular range of 5-50° (2θ) at room temperature. The Miller indices of synthetic vinyl-based and native oligocelluloses were assigned following the literature (Yataka et al., 2015).

2.3. In vitro synthesis of vinyl-based and native oligocelluloses using CtCdP

In a 50 ml falcon tube, α-Glc1P (1.82 g, 6.0 mmol) was dissolved in 30 ml HEPES buffer 500 mM, pH 7.5, at room temperature. Subsequently, 0.3 mmol of G-EM (83.4 mg), G-BA (96 mg), G-EEA (83.1 mg), G-EMAA (87.3 mg), or cellulobiose (102.7 mg) was added into the α-Glc1P solution. The reaction was started by adding the enzyme solution (250 μl) and putting the tube on an Eppendorf Thermomixer comfort (45 °C, 600 rpm). After few hours, the white turbid solution was observed. The reaction products were isolated by centrifugation on Thermo Scientific Heraeus Labofuge 400 R (4500 rpm, 20 min, 4 °C) and the precipitates were washed at least three times with Milli-Q water. The products were then lyophilized (−45 °C, 0.01 mbar) overnight. The product yields were calculated by comparing the isolated product weights with the theoretical product weights (the obtained Mw from MALDI-ToF MS measurements was used for the calculation of theoretical weights).

2.3.1. Oligocellulosyl-ethyl methacrylamide (OC-EMAAm)

White powder, 280 mg, yield: 70%. 1H NMR (4 wt% NaOD/D2O) δ in ppm: 5.51 (H11-cis), 5.26 (H11-trans), 4.26 (H2, J = 7.8 Hz), 4.18 (H1, J = 7.6 Hz), 3.03–3.81 (H3, H4, H5, H6, H7, H8, H9), 1.73 (H12).

2.3.2. Oligocellulosyl-ethyl acrylamide (OC-EAAm)

White powder, 248 mg, yield: 63%. 1H NMR (4 wt% NaOD/D2O) δ in ppm: 5.96-6.13 (H11-cis and H10), 5.56 (H11-trans, J = 11.6 Hz), 4.25 (H2, J = 8.0 Hz), 4.16 (H1, J = 8.0 Hz), 3.01–3.82 (H3, H4, H5, H6, H7, H8, H9).

2.3.3. Oligocellulosyl-ethyl acrylate (OC-EA)

White powder, 239 mg, yield: 58%. 1H NMR (4 wt% NaOD/D2O) δ in ppm: 5.74-5.91 (H11-cis and H10), 5.41 (H11-trans, J = 12 Hz), 4.21 (H2, J = 8.2 Hz), 4.14 (H1, J = 8.0 Hz), 2.97–3.67 (H3, H4, H5, H6, H7, H8, H9), 1.31–1.45 (H8′, H9′).

2.3.4. Oligocellulosyl-ethyl methacrylate (OC-EMA)

White powder, 298 mg, yield: 67%. 1H NMR (4 wt% NaOD/D2O) δ in ppm: 5.46 (H11-cis), 5.15 (H11-trans), 4.26 (H2, J = 7.8 Hz), 4.20 (H1, J = 8.0 Hz), 3.02–3.78 (H3, H4, H5, H6, H7, H8, H9), 1.67 (H12).

2.3.5. Oligocellulosyl-ethyl acrylate (OC-BA)

White powder, 304 mg, yield: 65%. 1H NMR (4 wt% NaOD/D2O) δ in ppm: 5.78-5.96 (H11-cis and H10), 5.45 (H11-trans, J = 11.6 Hz), 4.26 (H2, J = 8.0 Hz), 4.19 (H1, J = 7.6 Hz), 3.02–3.78 (H3, H4, H5, H6, H7, H8, H9).

2.3.6. Native oligocellulose (OC)

White powder, 231 mg, yield: 66%. 1H NMR (4 wt% NaOD/D2O) δ in ppm: 5.11 (Hα), 4.53 (Hβ, J = 7.2 Hz), 4.28 (H2, J = 8.2 Hz), 3.04–3.74 (H3, H4, H5, H6, H7, H8, H9).

2.4. Optimization reaction condition

Three 50 ml of falcon tubes were prepared and different amounts of α-Glc1P was added into each tube (0.91 g, 3.0 mmol; 1.82 g, 6.0 mmol; 3.64 g, 12.0 mmol) then dissolved by 30 ml HEPES buffer 500 mM, pH 7.5, at room temperature. Subsequently, G-EM (83.4 mg, 0.30 mmol) was added to the α-Glc1P solution. The reaction was started by adding the enzyme solution (250 μl) and putting the tubes in an Eppendorf Thermomixer comfort (45 °C, 600 rpm). After certain time intervals, TLC of the reaction mixture was performed and the reaction product was detected at retardation factor of 0.63.

3. Results and discussion

Celloctextrin phosphorylase (CdP, EC 2.4.1.49) is a member of the glycoside hydrolase family 94 and it is known to be able to catalyze both phosphorylase and synthesis of oligocelluloses in a stereospecific fashion (Kitaoka & Hayashi, 2002). CdP has high substrate promiscuity that gave us an opportunity to use not only its natural substrates but...
also unnatural substrates as the glucosyl acceptors in the reactions. For instance, Soetaert and coworkers reported the GdP from Clostridium stercorarium can catalyze the reaction with aryl- and alkyl-β-glucosides as well as glucos- and sophorolipids served as the substrate (Hai Tran, Desmet, De Groeve, & Soetaert, 2011; Tran et al., 2012). In our study, a recombinant GdP from Clostridium thermocellum (CtCdP) was employed to catalyze the synthesis of vinyl-based oligocelluloses via reverse phosphorylase mechanism as shown in Scheme 1(b). The enzymatic synthesis of vinyl-based oligocelluloses used vinyl glucosides as the glucosyl donors and the reaction was carried out in buffer media. The glucosides contain (meth)acrylate/(meth)acrylamide groups that are exclusively bond to the anomeric carbon of glucose at the beta configuration and these compounds were also synthesized enzymatically (Scheme 1(a)) using commercial β-glucosidase in aqueous environments as described before (Adharis et al., 2018; Kloosterman et al., 2014).

Five types of vinyl-based oligocelluloses were successfully synthesized from the corresponding vinyl glucosides: Oligocellulosyl-ethyl acrylate (OC-EA), oligocellulosyl-ethyl methacrylate (OC-EMA), oligocellulosyl-butyl acrylate (OC-BA), oligocellulosyl-ethyl acrylamide (OC-EAAm), and oligocellulosyl-ethyl methacrylamide (OC-EMAAm). Furthermore, we also synthesized native oligocellulose using cellulobiose as the natural substrate (Scheme 1(c)) in order to compare the characteristic of the synthesized vinyl-based oligocelluloses with the native ones. The transparent reaction mixtures upon catalysis by CtCdP became turbid, suggesting that water-insoluble products were formed during the synthesis of vinyl-based and native oligocelluloses. In contrast, the control reaction (without enzyme) remains transparent after 3 days confirming the role of the enzyme in the catalysis of the reactions. The reaction products were separated from the unreacted α-Glc1P and the biocatalyst by centrifugation and the precipitates were washed few times with water resulting in the isolated product yields from 58% to 70%.

Product formation of the enzymatic synthesis of OC-EA was followed by TLC using eluent mixtures of n-butanol/isopropanol/water. TLC analysis of the reaction mixtures was performed at different time intervals and different α-Glc1P concentrations. Fig. 2 shows that during the period of 72 h, spots belonging to the reaction product clearly appeared at a retardation factor of 0.63 whereas the spots of the glucosyl acceptor were diminished. According to the TLC results, all G-EA (10 mM) were completely reacted with α-Glc1P in the mentioned reaction conditions. Visual comparison of the product spots on TLC after 72 h reaction shows that the reaction condition with 200 mM α-Glc1P (Fig. 2(b)) resulted in a stronger spot intensity than the reaction condition with 100 mM (Fig. 2(a)). Besides this, the spots corresponding to the unreacted α-Glc1P were found to be more intense in the reaction with 400 mM (Fig. 2(c)) as compared with 200 mM (Fig. 2(b)). Based on these results, we concluded that the optimal reaction conditions were achieved when the concentration of glucosyl donor was twenty times the concentration of glucosyl acceptor. Therefore, this reaction condition was used for the synthesis of all other vinyl-based and native oligocelluloses.

3.1. Characterization of the synthesized vinyl-based oligocelluloses

Fig. 3(a) shows 1H NMR spectra of the enzymatically synthesized vinyl-based and native oligocelluloses with protons designated as in Scheme 1(b) and (c). Anomeric proton peaks of vinyl glucosides (H1) at 4.14-4.20 ppm and internal anomeric proton peaks (H2) of glucosyl repeating units at 4.21-4.26 ppm were clearly recognized, suggesting that the glucosyl units were successfully linked at the non-reducing end of the vinyl glucosides. In contrast to native oligocellulose, no α- and β-anomeric proton peaks (Hα & Hβ) were detected in the spectra of vinyl-based oligocelluloses. Furthermore, the proton peaks at 5.15-6.13 ppm correspond to vinyl protons (H10 & H11) of the substrates. Both results reveal that the alkyl-(meth)acrylate/(meth)acrylamide sequences continue to exist at the reducing end of the oligocelluloses after the enzymatic reaction.

The average degree of polymerization (DPn) of the vinyl-based oligocelluloses that equals to the average number of repeating glucosyl units, was obtained from the 1H NMR spectra by comparing the peak integration of both anomeric protons with one of the vinyl protons (see Eq. (3)) and the obtained DPn was in the range of 7.3-8.3, except for OC-EA (see below). The number-average molecular weight (Mn) of the prepared vinyl-based oligocelluloses was determined via Eq. (3) and the Mn’s are calculated to be between 1300 and 1500 g mol−1, except for OC-EA (Table 1). In addition, the DPn of native oligocellulose was 6.9, slightly lower than the vinyl-based oligocellulose based on the

Scheme 1. Enzymatic synthesis of (a) vinyl glucosides catalyzed by β-glucosidase, (b) vinyl-based and (c) native oligocelluloses catalyzed by CtCdP (m, A, and R are enlisted in Table 1).
calculation using Eq. (2). As a result, the $M_n$ of native oligocellulose was also lower than the vinyl-based oligocelluloses.

In the case of OC-EA, the calculated $M_n$ was about 3500 g mol$^{-1}$, 2.7 times higher than the other vinyl-based oligocelluloses. In our previous report (Petrović et al., 2015), higher $M_n$ of oligocellulose may be achieved during the enzymatic reaction by lowering the concentration of glucosyl acceptor which leads to lower concentration of the synthesized oligocellulose. Under this condition, the intermolecular hydrogen bond between oligocellulose can be reduced causing less precipitation and partially soluble oligocellulose can have further polymerization. Since this is not the case in this study, an error in peak integration of $^1$H NMR spectra used in Eq. (1) is the most possible reason for this anomalous result.

The intensity of vinyl proton peaks of OC-EA in Fig. 3 was much smaller than the intensity of vinyl proton peaks of other vinyl-based oligocelluloses, however, the intensity of internal anomeric proton peaks (H2) of those vinyl-based oligocelluloses was similar. Consequently, the amount of vinyl group of OC-EA is also lower than the other vinyl-based oligocelluloses but they have a comparable number of glucosyl repeating units. According to Eq. (1), if the amount of anomeric protons is constant but the amount of vinyl protons is decreasing, then the calculated $D_p$ will increase and the calculated $M_n$ will increase as well. The low amount of vinyl proton of OC-EA is possibly due to fragmentation of the acrylate unit that occurred during the enzymatic reaction.

In order to investigate whether the fragmentation of acrylate units of OC-EA is due to hydrolysis by NaOD during the preparation of $^1$H NMR samples, $^1$H NMR experiments of vinyl glucosides in the same conditions as vinyl-based oligocelluloses were performed and the results are shown in Fig. 3(b). Each vinyl glucosides still consisted of one (meth)acrylate/(meth)acrylamide groups after treating the samples in slightly basic condition according to the comparison of peak integration of the anomeric proton (H1) with the vinyl protons (H10, H11). The existing vinyl protons of acrylate units of G-EA indicated that no hydrolysis reaction occurred in the acrylate groups of G-EA as well as OC-EA during $^1$H NMR experiments.

The MALDI-ToF spectra of vinyl-based and native oligocelluloses are depicted in Fig. 4. The most dominant peaks of MALDI-ToF spectra of vinyl-based and native oligocelluloses were derived from the oligocellulose sequences with a number of repeating glucosyl units from 6 to 10. However, the most dominant peaks of MALDI-ToF spectrum of OC-EA (Fig. 4(b)) belong to the oligocellulose sequence with fragmentation on the alpha position of the acrylate unit, supporting the low intensity of the vinyl proton of OC-EA as witnessed from the $^1$H NMR experiment.

Even though the major fragmentation of OC-EA may also be due to the high energy laser irradiation during the MALDI-ToF MS experiments, we should see the same observation in the case of OC-BA since both of them have exactly the same acrylate group. In contrast, only a small amount of fragmented sequences were identified in the spectrum of OC-BA. Additionally, minor fragmentation was also observed in OC-EMA and no fragmentation in OC-EAAm, OC-EMAAm, and native oligocellulose. Therefore, the laser energy might only cause less/no
fragments. We suggest that this fragmentation phenomena in (meth)acrylate-based oligocelluloses happened due to a nucleophilic substitution of the phosphate ion to the (meth)acrylate groups during the enzymatic synthesis since CtCdP is capable to catalyze the phosphorolysis reaction as well. The proposed mechanism for this phosphorolysis reaction is shown in Scheme 2.

The contrary observation was reported by Freidig, Verhaar, and Hermens (1999) where methacrylates generally have a higher hydrolysis rate than acrylates at pH 7 in a conventional reaction. Considering the reaction center for hydrolysis and phosphorolysis of (meth)acrylate is exactly the same, it seems that the phosphorolysis in the enzymatic reaction also depends on the structures of the substrates that lead to different outcomes in comparison with the chemical reaction. The difference between the alkyl groups in the substrates (hydrogen vs methyl for G-EA and G-EMA; ethyl vs butyl for G-EA and G-BA) results in a different reactivity in the enzyme catalyzed phosphorolysis reaction. Furthermore, no fragmentation was discovered in the spectra of OC-EA and OC-EMA because of the (meth)acrylamide groups are well-known to be more stable towards nucleophilic substitution than (meth)acrylate groups. Indeed, the study on the selectivity of this enzyme with di (meth)acrylate-based oligocelluloses happened due to a nucleophilic substitution of the phosphate ion to the (meth)acrylate groups during the enzymatic synthesis since CtCdP is capable to catalyze the phosphorolysis reaction as well. The proposed mechanism for this phosphorolysis reaction is shown in Scheme 2.

Table 1
Overview of the enzymatically synthesized vinyl-based and native oligocelluloses.

<table>
<thead>
<tr>
<th>Substrate names</th>
<th>m/A/R</th>
<th>Product names</th>
<th>MALDI-ToF MS</th>
<th>SEC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td>$M_n$, $M_w$, PDI</td>
<td>$M_n$, $M_w$, PDI</td>
</tr>
<tr>
<td>G-EMAAm</td>
<td>1/NH/CH$_3$</td>
<td>OC-EMAAm</td>
<td>1435 8.1</td>
<td>1326 1443 1.09 7.4</td>
</tr>
<tr>
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<td>1/NH/H</td>
<td>OC-EAam</td>
<td>1335 7.5</td>
<td>1310 1323 1.01 7.4</td>
</tr>
<tr>
<td>G-BA</td>
<td>2/O/H</td>
<td>OC-BA</td>
<td>1327 7.3</td>
<td>1385 1413 1.02 7.7</td>
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<tr>
<td>G-EA</td>
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<td>OC-EA</td>
<td>1475 8.3</td>
<td>1492 1520 1.02 8.4</td>
</tr>
<tr>
<td>G-EE</td>
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<td>OC-EE</td>
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<td>1553 1647 1.06 8.9</td>
</tr>
<tr>
<td>Cellulose</td>
<td>–</td>
<td>OC</td>
<td>1138 6.9</td>
<td>1170 1195 1.02 7.1</td>
</tr>
</tbody>
</table>

Number-average molecular weight ($M_n$) and weight-average molecular weight ($M_w$) in gram mol$^{-1}$. 

Fig. 4. (a) MALDI-ToF MS spectra of the synthesized vinyl-based and native oligocelluloses catalyzed by CtCdP. Asterisk symbols in OC-EA, OC-EMA, and OC-BA show the signals that relate to the fragmented oligocelluloses. (b) Magnification of OC-EA and OC-EMA peaks of the MALDI-TOF MS spectra.
SEC is lower than the vinyl-based ones, in agreement with the result obtained from $^1$H NMR and MALDI-ToF experiments (Table 1). WAXD experiments were performed to determine the crystal type of the synthetic vinyl-based and native oligocelluloses. Cellulose exists in several crystal lattices namely cellulose I, II, III, and IV where each polymorph has different unit cell parameters (Wertz, Bédué, & Mercier, 2010). WAXD profile of both vinyl-based and native oligocelluloses (Fig. 6) exhibits exactly the same pattern with three reflection peaks at $2\theta$ of around 12.2° ($d = 7.26 \text{ Å}$), 19.8° ($d = 4.48 \text{ Å}$), and 22.0° ($d = 4.04 \text{ Å}$). A similar observation was also reported in the literature (Hiraishi et al., 2009; Yataka et al., 2015). This result concludes that our vinyl-based and native oligocelluloses follow the cellulose II polymorph, the most thermodynamically stable form of crystalline cellulose.

The ordered structure of the synthesized oligocelluloses is a result of the strong intermolecular hydrogen bonds during enzymatic synthesis. Furthermore, it is shown that different types of end group functionalities do not affect the crystal lattice of the oligocelluloses.

4. Conclusion

We have successfully synthesized five types of well-defined vinyl-based oligocelluloses catalyzed by CtCdP in buffer solution. The enzymatic synthesis was followed by TLC and the products were identified at a retardation factor of 0.63. The optimum product formation was reached when the concentration of glucosyl donor is twenty-fold of the glucosyl acceptors. The prepared vinyl-based oligocelluloses possess $D_P$ and $M_w$ of 7.3–8.9 and 1310–1553 g mol$^{-1}$, respectively, according to $^1$H NMR, MALDI-ToF MS, and SEC measurements. Fragmentation phenomena at the alpha position of (meth)acrylate units was observed in OC-EA, OC-EMA, and OC-BA but this observation was absent in OC-EAAm, OC-EMAAm, and native OC. Furthermore, based on the WAXD experiments the synthesized vinyl-based and native oligocelluloses belong to the cellulose II polymorph.

The synthesis of vinyl-based oligocelluloses and the precursors were successfully conducted through eco-friendly pathways. In addition, the CtCdP was presented to have substrate promiscuity with different vinyl glucosides other than its natural substrate. Unfortunately, the commercial availability of CtCdP, the loss of CtCdP during purification, and the cost of the glucosyl donor, $\alpha$-Glc1P, are still challenges for future commercialization. Moreover, further experiments will be directed to prepare the (co)polymers of these vinyl-based oligocelluloses and to study their application for thermoresponsive materials, novel bio-based...
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References


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surfactants, and so forth. In order to improve the solubility of oligo-celluloses in solution, ionic liquids may be used as suitable green solvents (Wang, Gurau, & Rogers, 2012).

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