Alternating Copolysaccharides

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Glucose/Glucuronic Acid Alternating Co-polysaccharides Prepared from TEMPO-Oxidized Native Celluloses by Surface Peeling**

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Alternating co-polysaccharides (ACPS) have regular molecular structures that consist of repeating hetero-disaccharide units. ACPS consisting of neutral and uronate sugars, such as naturally occurring hyaluronan, have unique bioactive properties including antiinflammatory, wound healing, and moisture retention behavior.[1] However, the artificial synthesis of ACPS is generally difficult and requires multistep protection/deprotection reactions owing to the multi-hydroxy structures of the mono- or disaccharide starting materials that result in low molecular weight and low yield of ACPS products.[2] Partial or complete enzymatic processes are often successful in synthesizing ACPS including hyaluronan-type co-polysaccharides; although these processes require long reaction times, multistep derivatizations of the starting mono- or disaccharides for activation, and high purity of the starting materials.[3]

Another candidate starting material for the preparation of ACPS is the use of naturally occurring and highly crystalline polysaccharides such as cellulose. When 2,2,6,6-tetramethylpiperidin-1-yloxyl (TEMPO)-mediated oxidation is applied to native celluloses consisting of crystalline microfibrils of cellulose I, the primary hydroxy groups at C6 exposed on the microfibril surfaces are entirely oxidized to sodium carboxylate groups in a selective manner, thus maintaining the original cellulose I crystal structure, crystallinity index, crystal size, and microfibril morphology.[4] Thus, every one of two glucosyl residues of the extended cellulose chains present on the crystalline microfibril surfaces can be converted into a sodium glucuronosyl residue by TEMPO-mediated oxidation.[4e]

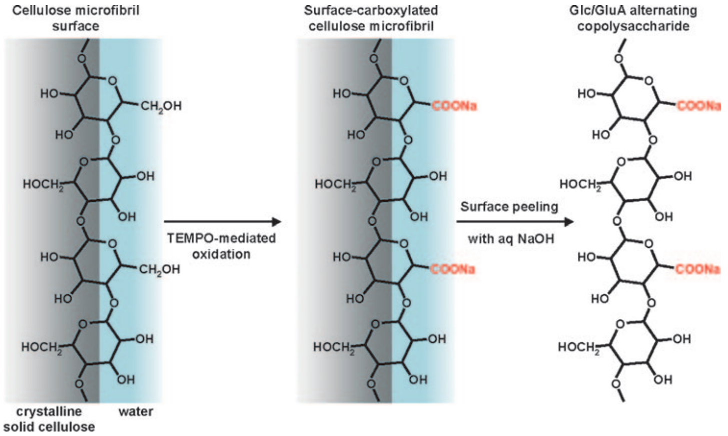

Figure 1. Schematic model for the preparation of glucose/glucuronic acid (Glc/GluA) alternating co-polysaccharide by surface peeling of TEMPO-oxidized native celluloses with aqueous NaOH.

Based on this background, an attempt was made to prepare glucose/glucuronic acid ACPS linked with (1→4)-β-glycoside bonds by surface-peeling of TEMPO-oxidized native cellulose with a solution of 20% aqueous NaOH. Cellulose molecules are insoluble in water; therefore, only the glucose/glucuronic acid alternating co-polysaccharide molecules should be obtained as a water-soluble fraction from the 20% aqueous NaOH extract after neutralization with acid (Figure 1).

A commercially available bleached softwood kraft pulp containing 90% cellulose and 10% noncellulosic polysaccharides was used as the wood cellulose fiber. Tunicate cellulose of *Halocynthia roretzi* was purified and milled to fine gel-like particles in water by mechanical disintegration using a household blender. TEMPO-mediated oxidation of tunicate and wood celluloses was carried out in water at pH 10 using a TEMPO/NaBr/NaClO system according to the reported method.[4,5] The amount of NaClO added was either 5 or 10 mmol per gram of dry cellulose.[5]

The respective fibrous and fine gel-like particle morphologies of the wood and tunicate celluloses were maintained after TEMPO-mediated oxidation. The TEMPO-oxidized native celluloses suspended in water (0.15% w/v) were then sonicated to prepare highly viscous and transparent gels (Figure 2a).[5] The apparently transparent gels consisted of highly crystalline and individualized TEMPO-oxidized tunicate and wood cellulose nanofibrils of about 4 nm and 9 nm in width, respectively,[4e] so that the dispersions exhibited birefringence when observed between cross polarizers.[6] The TEMPO-oxidized cellulose nanofibril/water dispersion

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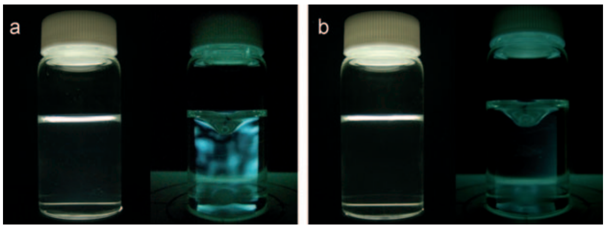

Figure 2. TEMPO-oxidized wood cellulose nanofibril dispersions at 0.1% solid content in a) water and b) 20% aqueous NaOH, observed with (right) and without (left) cross-polarizers.

(100 mL) was then slowly poured into a solution of 40% aqueous NaOH (100 g). Even though the apparently transparent dispersions were maintained, birefringence was no longer observed owing to the destruction of the crystalline nanofibril structures in a solution of 20% aqueous NaOH (Figure 2b).

After neutralization of the solutions of 40% aqueous NaOH with 10 M aqueous acetic acid, the aqueous fractions were separated, and water-soluble polysaccharides were obtained as precipitates after addition of ethanol and successive purification.[5] The yield, carboxylate content, weight average molecular mass (Mw), and the corresponding degree of polymerization (DPw) of the water-soluble products were measured,[5] and are listed in Table 1.

Table 1: Weight recovery ratio, carboxylate content, weight average molecular mass (M_w), and the corresponding weight average degree of polymerization (DP_w) for the water-soluble fractions in the 20% aqueous NaOH extracts of TEMPO-oxidized tunicate and wood celluloses prepared with 5 mmol NaClO per gram of cellulose.[5]

Sample	Recovered mass [wt%][a]	Carboxylate content [mmol g^{-1}]	M_w (DP_w)
TEMPO-oxidized tunicate cellulose		0.57	–
water-soluble fraction	6.9	2.57	7400 (41)
water-insoluble residue[b]	87.8	0.37	–
TEMPO-oxidized wood cellulose		1.48	–
water-soluble fraction	27.4	2.66	11 000 (61)
water-insoluble residue[b]	55.8	0.91	–

[a] Based on the weight of TEMPO-oxidized cellulose. [b] The water-insoluble fraction in 20% NaOH extract combined with the water-insoluble fraction in 20% NaOH.

The major differences between the tunicate and wood celluloses are crystallinity (ca. 95% and 65%, respectively), crystal width (9 and 4 nm, respectively),[4e] and crystal structures (almost pure cellulose I_β and low-crystalline cellulose I_β-rich structures, respectively).[7] The carboxylate contents of the TEMPO-oxidized tunicate and wood celluloses before extraction with 20% aqueous NaOH were 0.57 and 1.48 mmol g^{-1}, respectively (Table 1). These carboxylate contents were consistent with the amounts of primary hydroxy groups at C6 that were exposed on the microfibril surfaces, which were calculated based on the cellulose microfibril widths determined from X-ray diffraction.[4e] Accordingly, the primary hydroxy groups at C6 present on the microfibril surfaces of each native cellulose were oxidized to carboxylate groups.

Because the fractions separated from the water-soluble products still had carboxylate groups of 0.37 and 0.91 mmol g^{-1} for TEMPO-oxidized tunicate and wood celluloses (Table 1), respectively, complete extraction of the carboxylate-containing fractions could not be achieved by treatment with 20% aqueous NaOH at room temperature. However, longer extraction times or higher extraction temperatures resulted in lower M_w values and some side reactions, including β elimination.[8] When all the primary hydroxy groups (at C6) of cellulose are oxidized to sodium carboxylate groups, the oxidized products, that is, cellouronic acid Na salt or (1→4)-β-D-polyglucuronate Na salt, should have a carboxylate content of 5.05 mmol g^{-1}. Because the carboxylate content of the water-soluble products in Table 1 was approximately half that of cellouronic acid Na salt, these water-soluble products had glucosyl and sodium glucuronosyl residues in a ratio of approximately 1:1 by mol.

The ^{13}C NMR spectra of the water-soluble products and the corresponding signal assignments are presented in Figure 3. These assignments were achieved from ^{1}H and ^{13}C NMR, double quantum filter correlation (DQF-COSY), heteronuclear single quantum coherence (HSQC), and constant-time heteronuclear multiple-bond correlation (CT-HMBC) spectroscopic analysis of the products.[5] The ^{13}C signals intensities corresponding to C4 and C4a were quite similar to each other. This observation also indicates that the obtained water-soluble products consist of glucosyl and

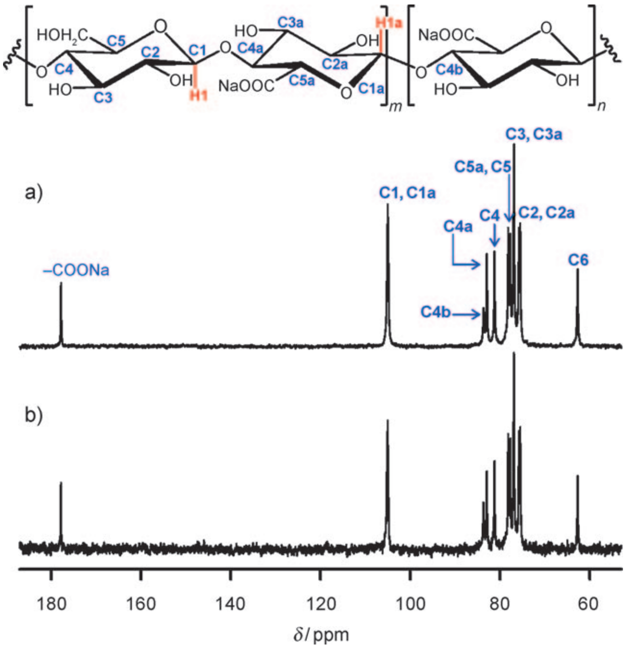

Figure 3. ^{13}C NMR spectra of water-soluble products in 20% aqueous NaOH extracts of TEMPO-oxidized a) tunicate and b) wood celluloses, with corresponding signal assignments.

sodium glucuronosyl residues alone with a ratio of about 1:1 per mol, and these are linked by (1→4)-β-glycoside bonds. The key issue is whether the two sugar residues form a regularly alternating, a block, or a random co-polysaccharide structure. The CT-HMBC spectrum revealed that the signal corresponding to C4a of the sodium glucuronosyl residue had no correlation with H1a, but had a clear correlation with H1 of the glucosyl residue.[5] These long-range interactions clearly showed that the residue adjacent to the glucosyl residue was always the sodium glucuronosyl residue. Therefore, it was concluded that glucose/glucuronic acid ACPS with (1→4)-β-glycoside bonds can be prepared by surface peeling of the TEMPO-oxidized native cellulose microfibrils with 20% aqueous NaOH, according to what was outlined in Figure 1.

A small signal corresponding to C4b of the (1→4)-β-D-homopolyglucuronic acid Na salt moiety was observed in both the ^{13}C NMR spectra shown in Figure 3. Approximate calculations showed that the ratio *m/n* was 6.5:1 and 4.5:1 for the TEMPO-oxidized tunicate and wood celluloses, respectively; the water-soluble products contained about 7% and 10% of the homopolyglucuronic acid Na salt moieties, respectively, probably at the end(s) of each molecular chain.[5] Therefore, wood cellulose microfibrils have somewhat more disordered or imperfectly crystalline structures on the surfaces that may be converted into the homopolyglucuronic acid Na salt-type structure by TEMPO-mediated oxidation. The *m/n* values and the data in Table 1 resulted from different surface structures of the two native cellulose microfibrils, details of which were discussed in the last paragraph of the Supporting Information.[5]

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