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Degrees of polymerization (DP) and DP distribution of cellouronic acids prepared from alkali-treated celluloses and ball-milled native celluloses by TEMPO-mediated oxidation

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Abstract Various cellulose II samples, ball-milled native celluloses and ball-milled wood saw dust were subjected to 2,2,6,6-tetramethypyperidine-1-oxyl radical (TEMPO)-mediated oxidation to prepare cellouronic acid Na salts (CUAs). The TEMPO-oxidized products obtained were analyzed by ^{13}C-NMR and size-exclusion chromatography (SEC). When the cellulose II samples with degrees of polymerization (DP) of 220–680 were used as the starting materials, the CUAs obtained had weight-average DP (DPw) values of only 38–79. Thus, significant depolymerization occurs on cellulose chains during the TEMPO-mediated oxidation. These DP values of CUAs correspond to the cellulose II crystal sizes along the chain direction in the original cellulose II samples, but not necessarily to their leveling-off DP values. CUAs can be obtained also from ball-milled native celluloses in good yields by TEMPO-mediated oxidation, although their DPw values are lower than about 80. On the other hand, CUA with DPw of about 170 was obtained from ball-milled wood saw dust.

Keywords TEMPO · Cellouronate · Cellouronic acid · Ball-milling · Mercerized cellulose · Regenerated cellulose · Molecular mass · SEC-MALLS · LODP

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Introduction

We have studied 2,2,6,6-tetramethyl-piperidine-1-oxyl radical (TEMPO)-mediated oxidation of celluloses in aqueous solutions around pH 10 to prepare water-soluble β-(1 → 4)-linked polyglucuronic acid sodium salt (cellouronic acid or CUA). When regenerated and mercerized celluloses are used as the starting materials, CUAs are obtained quantitatively (Isogai and Kato 1998). However, remarkable decreases in degree of polymerization (DP) occur on the cellulose molecules during the oxidation, and CUA prepared from viscose rayon (DPv 380) has DP of only about 40 (Shibata et al. 2006). This DP value corresponds well to the leveling-off DP (LODP) values of regenerated celluloses, which were obtained by dilute acid hydrolysis at high temperatures (Battista et al. 1956; Schurz and John 1975; Yachi et al. 1983; Fan et al. 1987; Einfeldt et al. 2005; Isogai et al. 2008) and by small-angle neutron diffraction analysis combined with the deuterium-exchange method (Fischer et al. 1978). Thus, depolymerization of disordered regions in viscose rayon seems to occur preferably during the TEMPO-mediated oxidation.

The following two depolymerization mechanisms of polysaccharides including cellulose during the oxidation have been proposed so far: (1) β-elimination of glycoside bonds at pH 10–11 due to C6-aldehyde groups formed as intermediates during the oxidation (de Nooy et al. 1996; Potthast et al. 2007), and (2) some hydroxyl radicals formed in situ as by-products during

the oxidation (Shibata and Isogai 2003). Because significant depolymerization occurs also on once-prepared CUA molecules under the oxidation conditions, the latter as well as the former probably plays an important role in the remarkable depolymerization of CUAs.

As described above, because the DP value of CUA prepared from viscose rayon corresponds well to its LODP, the higher the LODP values of the starting cellulose samples are, the higher the DP values of CUAs prepared thereof by TEMPO-mediated oxidation are expected to be. In a previous paper (Isogai et al. 2008), we determined LODP values of 20% NaOH-treated native, regenerated and 20% NaOH-treated regenerated celluloses (M-native, regenerated and M-regenerated celluloses, respectively) by size-exclusion chromatography furnished with a multi-angle laser-light scattering detector (SEC-MALLS) of their hydrolyzed products, which were prepared from the cellulose II samples by acid hydrolysis with 1 M HCl at 105 °C for 1–3 h. Because the LODP values of M-native, regenerated and M-regenerated celluloses are 64–66, 35–49 and 60–74, respectively, CUAs with DP around 60–74 are expected to be prepared, when M-native and M-regenerated celluloses are used as the starting materials in TEMPO-mediated oxidation. In addition, the above LODP values of regenerated and M-regenerated celluloses indicate that the cellulose II crystal sizes of regenerated celluloses increase to the cellulose chain direction by the 20% NaOH treatment. It was also found that every SEC elution pattern of the acid-hydrolyzed products prepared from the cellulose II samples examined had a fraction corresponding to DP 20 as a minor component. If these components are present as minor crystalline regions in the original cellulose II samples, CUAs are plausible to have such DP fractions in their SEC elution patterns.

In this paper, first CUAs are prepared from M-native, regenerated and M-regenerated celluloses by TEMPO-mediated oxidation to compare their DP values with those obtained by dilute acid hydrolysis. Second, native celluloses and wood saw dust are ball-milled to convert the crystalline cellulose I regions in the native celluloses to disordered ones (Hermans and Weidninger 1946), and were subjected to TEMPO-mediated oxidation to prepare CUAs with higher DP values. Recently, some new and effective ball-milling apparatuses have become commercially available, and efficiencies of enzymatic hydrolysis and chemical modifications of cellulose are improved by using ball-milled celluloses (Tassinai and Macy 1977; Ryu et al. 1982; Endo et al. 1999; Mais et al 2002; Qiu et al. 2005). Because native celluloses have highly crystalline cellulose I structures, no water-soluble CUAs can be obtained by TEMPO-mediated oxidation even under harsh conditions (Isogai and Kato 1998). In this case, only the C6 primary hydroxyl groups present on the surfaces of crystalline cellulose microfibrils are oxidized to C6 carboxylate groups (Saito and Isogai 2004; Saito et al. 2006, 2007). Thus, some pretreatment of native celluloses is required to prepare CUAs in good yields. Liquid NH_3 treatment of native cellulose to convert cellulose I to III with low crystallinity has been proposed so far as a pretreatment to prepare CUAs by TEMPO-mediated oxidation (Da Silva Perez et al. 2003; Habibi and Vignon 2008). CUAs with DP 40–130 in 8–80% yields were obtained by using the NH_3-pretreated native celluloses (microcrystalline cellulose, cotton linters, microbial cellulose, softwood bleached kraft pulp etc.) as the starting materials in TEMPO-mediated oxidation. On the other hand, because no solvents or chemicals are needed in ball-milling of native celluloses, CUAs with higher DP values are expected to be prepared from ball-milled native celluloses or wood saw dust by TEMPO-mediated oxidation in this study.

Experimental

Materials

Cotton linters (Ash-less filter pulp, Advantec Tokyo Co., Japan) and fibrous microcrystalline cellulose (CF11, Whatman Internationals, Ltd., UK) were used as native celluloses. Tencel (regenerated cellulose fiber prepared from dissolving wood pulp using the N-methylmorpholine-N-oxide system, Lenzing Co., Austria) and Bemliese (regenerated cellulose fiber prepared from cotton linters using the cuprammonium solvent system) were used as regenerated celluloses. Native and regenerated celluloses were soaked in 20% NaOH at room temperature for 1 day. The alkali-treated native and regenerated celluloses were then washed thoroughly with water by filtration using a glass filter, and the corresponding freeze-dried samples were abbreviated as M-native and M-regenerated

celluloses, respectively. A softwood saw dust sample (Japanese cedar, *Cryptomeria japonoca*) was ball-milled for 4 h at room temperature to prepare fine wood powder with non-crystalline cellulose by means of a laboratory vibration mill with alumina balls (MB-1, Chuo Kakoki Co. Japan). Cotton linters and CF11 were also pulverized at room temperature up to 24 h in a container with balls made of agate using a planetary ball mill (P-7, Fritsch Co. Japan). A regenerated cellulose II sample with DP 15 was prepared from CF11 by dissolution in 85% H_3PO_4 followed by regeneration in water (Isogai and Usuda 1991). TEMPO, sodium bromide, 9 mass % sodium hypochlorite solution and other chemicals and solvents were of laboratory grade (Wako Pure Chemicals, Co. Japan), and used without further purification.

TEMPO-mediated oxidation

Cellulose or wood powder (1 g) was suspended in water (100 mL) containing TEMPO (0.025 g) and sodium bromide (0.25 g), and the oxidation was started by adding the NaClO solution to the slurry at room temperature and pH 10. The amounts of the 9% NaClO solution added were 10–15 mL, which corresponded to 12–18 mmol NaClO. During the oxidation, the slurry was always adjusted to pH 10 with a 0.5 M NaOH using a pH stat. Almost transparent solutions were obtained within 2 h for all cellulose and wood samples by the oxidation. The reaction was then quenched by adding a small amount of ethanol to the mixture, and the solution was centrifuged at 10,000*g* for 10 min to remove insoluble particles, when they were present. A large amount of ethanol was then added to the transparent solution, and the precipitate thus formed was collected by centrifugation. After being washed thoroughly with 80% aqueous ethanol by repeated centrifugation, the ethanol-containing product was dissolved in water. The solution was evaporated to remove residual ethanol, and the TEMPO-oxidized product was then obtained as a white solid by freeze-drying of the aqueous solution.

SEC-MALLS analysis

The TEMPO-oxidized product (1 mg) was dissolved in 0.1 M NaCl (1 mL), and the solution was filtered in two stages using 0.2 μm polytetrafluoroethylene (PTFE) membrane (Milex-LG, Milipore, USA) and then 0.02 μm PTFE disposable membrane (Anotop 25, Whatman, UK). Molecular mass parameters of the oxidized products were measured by means of a size-exclusion chromatograph (SEC) furnished with a multi-angle laser light-scattering detector (MALLS: DAWN EOS, $\lambda = 685$ nm, Wyatt Technologies, U.S.A) using 0.1 M NaCl as an eluent. A polyhydroxymethacrylate gel (SB-804, 8 mm $\phi \times 30$ cm, Shodex, Japan) was used as the SEC column. The cells of MALLS and refractive index detectors were kept at room temperature and 40 °C, respectively. Details of the SEC-MALLS system and operation conditions were described elsewhere (Shibata et al. 2006). The value of 0.125 mL/g was used as the specific refractive index increment (*dn/dc*) of CUA in 0.1 M NaCl (Shibata et al. 2006). Data acquisition and processing was performed with the ASTRA software (Wyatt Technologies).

Other analyses

Viscosity average degrees of polymerization (DPv) of ball-milled native celluloses were determined using 0.5 M cupriethylene diamine (Isogai et al. 1989). Solution-state ^{13}C-NMR spectra of the TEMPO-oxidized products were recorded on a JEOL Alpha 500 spectrometer (JEOL, Japan) using D_2O and trimethylsilyl propionic acid Na salt (both from Wako Pure Chemicals Co., Japan) as the solvent and internal standard, respectively. The original cellulose samples before the oxidation were converted to pellets using a KBr disk apparatus for FT-IR analysis. The pellets were subjected to X-ray diffraction measurement by the reflection mode using a Rigaku RINT 2000 with monochromatic CuKα radiation at 40 kV and 40 mA. The crystallinity indices of cellulose I in the ball-milled native celluloses were determined from the ratio of the separated peak area due to cellulose I to the total area from 5° to 35° of 2θ (Wada et al. 2004).

Results and discussion

In this study, various cellulose II samples, ball-milled native celluloses and ball-milled wood saw dust were used as the starting materials for preparing CUAs by TEMPO-mediated oxidation in water at pH 10. ^{13}C-

NMR spectroscopy was used to confirm the obtained oxidized products to be CUAs, and SEC-MALLS analysis was adopted to determine weight-average and number-average degrees of polymerization (DPw and DPn, respectively) and DP distributions of the oxidized products prepared from different origins. Figure 1 shows the main experimental scheme of the CUA preparation from various cellulose and wood samples, and characterization of the CUAs thus obtained.

Preparation of CUA from cellulose II samples

Four categories of cellulose II samples were used as the starting materials in the TEMPO-mediated oxidation: 20% NaOH-treated native celluloses (M-native celluloses), commercial regenerated cellulose fibers, those treated with 20% NaOH (M-regenerated celluloses) and the regenerated cellulose II with DP 15. Yields after the 20% NaOH treatment, crystallinity indices and other detailed information of the cellulose II samples used were described in the previous paper (Isogai et al. 2008). X-ray diffraction patterns of M-native, regenerated, M-regenerated celluloses and the regenerated cellulose II with DP 15 were shown in Fig. 2. All the samples had the cellulose II crystal structure, while they had different crystallinity indices and crystal sizes.

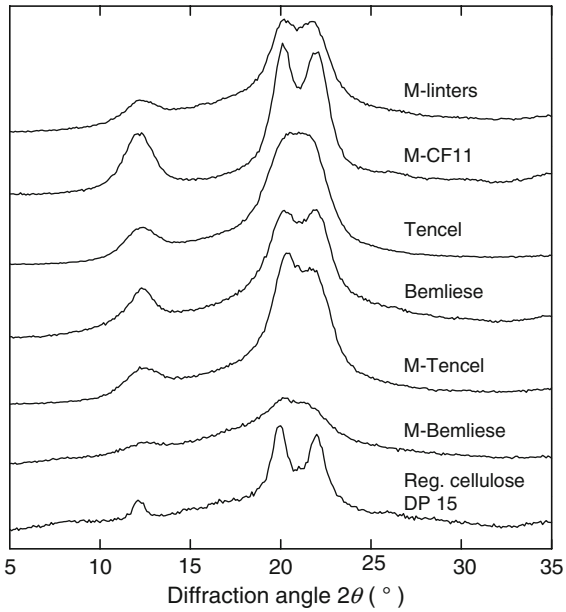

Fig. 2 X-ray diffraction patterns of cellulose II samples used in TEMPO-mediated oxidation

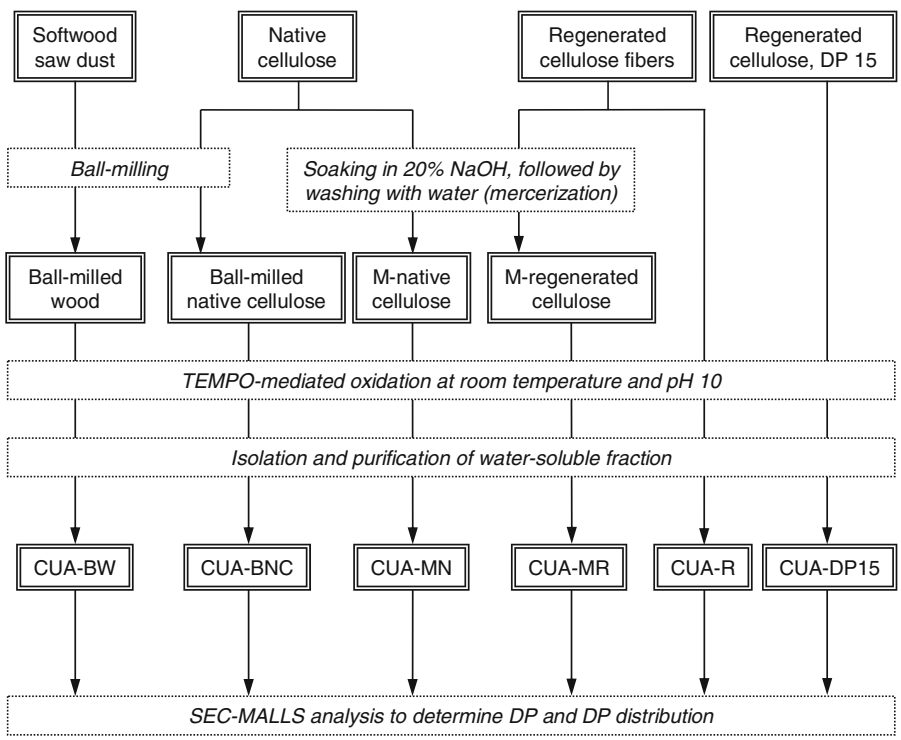

Fig. 1 Experimental scheme of SEC-MALLS analysis of cellouronic acids (CUA) prepared from various cellulose II samples, ball-milled native celluloses and ball-milled wood saw dust by TEMPO-mediated oxidation

Water-soluble products were obtained from the cellulose II samples in Fig. 2 by the TEMPO-mediated oxidation within 2 h, irrespective of the crystallinity indices, crystal sizes or DPv values of the starting cellulose II samples. Solution-state ^{13}C-NMR spectra showed that all the water-soluble products thus obtained had the chemical structure of CUA, which consisted of the β-(1 → 4)-linked glucuronic acid Na salt unit; all C6 primary hydroxyl groups of the cellulose II samples were converted to C6 carboxylate groups by the TEMPO-mediated oxidation (Isogai and Kato 1998). SEC elution patterns and molecular-mass (MM) plots of the CUAs prepared from the cellulose II samples in Fig. 2 are depicted in Fig. 3. Every SEC elution pattern has normal distribution without any shoulders. The peak top positions in Fig. 3 vary, depending on the cellulose samples used as the starting materials. The CUA prepared from the regenerated cellulose II with DP 15 has a peak top position at clearly higher elution volume or lower molecular-mass value than those of the others. The MM plots mostly lay on the same line, indicating that CUA molecules are properly separated by the SEC column according to their radii of gyration.

The DPv values of the original cellulose II samples, and yields and DP values of the CUAs prepared thereof by the TEMPO-mediated oxidation are listed in Table 1. Yields of CUAs were in the range of 84–94%, and the yield losses were primarily caused by handling during the isolation and purification processes. Except for the CUA prepared from the regenerated cellulose II with DP 15, the DPw values

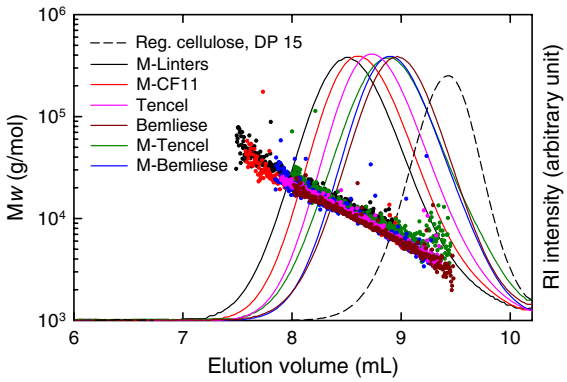

Fig. 3 SEC elution patterns and the corresponding molecular mass plots of cellouronic acids prepared from various cellulose II samples in Table 1 by TEMPO-mediated oxidation

Table 1 Yield, DPw, DPn and DPw/DPn values of cellouronic acid Na salt (CUA) prepared from various cellulose II samples by TEMPO-mediated oxidation

Cellulose II sample	Original DPv	CUA			
		Yield (%)	DPw	DPn	DPw/DPn
M-linters	410	87	79	57	1.39
M-CF11	220	84	55	41	1.34
Tencel	380	88	49	34	1.44
Bemliese	680	94	38	27	1.41
M-Tencel	450	89	45	32	1.41
M-Bemliese	380	90	38	28	1.36
Regenerated cellulose II	15	84	16	15	1.07

of CUAs were 38–79, which are clearly lower than the DPv values (220–680) of the original cellulose II samples. Thus, significant depolymerization of cellulose chains occurs during the oxidation. The DPw values of the CUAs prepared from M-linters and M-CF11 were slightly but clearly higher than those of CUAs prepared from regenerated or M-regenerated celluloses. No clear differences in DPw values of CUAs were observed between Tencel and M-Tencel or between Bemliese and M-Bemliese used as the starting materials. However, the CUAs prepared from Tencel and M-Tencel had clearly higher DPw values than those of CUAs prepared from Bemliese and M-Bemliese. These results indicate that the DP values of CUAs do not depend on those of the original cellulose II samples but are probably governed by longitudinal crystal sizes of cellulose II, whose information can be obtained as LODP values by dilute acid hydrolysis. These subjects are discussed in the following section. The polydispersity values (DPw/DPn) of CUAs were similar to each other, and ranged from 1.34 to 1.44 except for the regenerated cellulose II with DP 15.

Differences in DP values between CUAs and acid-hydrolyzed products prepared from cellulose II samples

The DP values of CUAs prepared from the cellulose II samples except the regenerated cellulose II with DP 15 in Table 1 may reflect solid-state structures or distribution of disordered regions along one cellulose chain in the cellulose II samples. In fact, the highly

crystalline cellulose II sample with DP 15, which might have no disordered regions at least without both ends in one cellulose chain, was converted to CUA with the same DP value as that of the original cellulose; the lengths of cellulose chains in the crystalline regions are mostly maintained without depolymerization during the TEMPO-mediated oxidation. On the other hand, disordered regions, when they are present in the intermediate positions along one cellulose chain in the solid cellulose II samples, should be preferably depolymerized by β-elimination at C6 aldehyde groups formed as intermediates at pH 10 (de Nooy et al. 1996; Potthast et al. 2007) and/or by some radical species formed in situ during the TEMPO-mediated oxidation (Shibata and Isogai 2003). Here, the rate of depolymerization of the disordered regions in the cellulose II samples might be much higher than that of the oxidation of C6 primary hydroxyl groups in the crystalline regions; the disordered regions in the cellulose II samples are first depolymerized to form microcrystals corresponding to the LODP values, and then water-soluble CUA molecules are formed and peeled off from the microcrystal surfaces by TEMPO-mediated oxidation. Thus, the DP values of CUAs in Table 1 may correspond to LODP values, which are observed for cellulose II samples by dilute acid hydrolysis at high temperatures. The LODP behavior has been thought to be reflected by crystal sizes along the longitudinal direction of cellulose chains present in the original celluloses before acid hydrolysis.

In the previous paper, the cellulose II samples in Table 1 were hydrolyzed with 1 M HCl at 105 °C for 1–3 h, and their DP values of the hydrolyzed products were determined by SEC-MALLS using LiCl/1,3-dimethyl-2-imidazolidinone as the solvent and eluent (Isogai et al. 2008). Table 2 shows the average peak top DP values in the SEC elution patterns of the dilute-acid hydrolyzed products of the cellulose II samples and those of CUAs prepared in this study. Every hydrolyzed product had a bimodal SEC elution pattern with a large main peak at DP 40–80 and a small shoulder around DP 20, whereas every CUA had a single SEC elution pattern with the peak top position of DP 34–57 without any shoulder peaks.

Because the main peak DP values in the SEC elution patterns of the dilute acid-hydrolyzed products of regenerated celluloses clearly increased from 40 to 80 by the 20% NaOH treatment (Table 2), CUAs with higher DP values than 40 were expected to be obtained by the TEMPO-mediated oxidation of M-regenerated celluloses instead of regenerated celluloses. However, the main peak DP values of CUAs prepared from M-regenerated celluloses were almost the same as those of CUAs prepared from regenerated celluloses. These results indicate that the cellulose II crystal sizes along the chain direction are intrinsically similar between regenerated and M-regenerated celluloses. To rationally explain the discrepancy in the DP values between CUAs and acid-hydrolyzed products prepared from the same M-regenerated celluloses in Table 2, the following hypothesis is

Table 2 Average peak-top DP values in SEC elution patterns of acid hydrolyzed products and cellouronic acid Na salts prepared from various cellulose II samples by dilute acid hydrolysis at 105 °C for 3 h and TEMPO-mediated oxidation at room temperature and pH 10 for 2 h, respectively

Cellulose II samples	Dilute acid hydrolysis at 105°C for 3 h[a]	TEMPO-mediated oxidation at room temp. and pH 10
20% NaOH-treated native celluloses	20 (22%) + 69 (78%)	57
Regenerated celluloses	19 (15%) + 40 (75%)	40
20% NaOH-treated regenerated celluloses	22 (35%) + 80 (65%)	34

[a] Data from the previous paper (Isogai et al. 2008)

applicable to the LODP behavior of M-regenerated celluloses. The cellulose II crystal sizes along the chain direction are almost the same between regenerated and M-regenerated celluloses, thus resulting that the similar DP values of CUAs are obtained from both of them by the TEMPO-mediated oxidation. On the other hand, the increase in the cellulose II crystal sizes along the chain direction occurs only during the acid hydrolysis process of M-regenerated celluloses with 1 M HCl at 105 °C. The occurrence of the increase in the cellulose II crystal sizes are probably associated with depolymerization in the disordered regions and some cellulose chain rearrangements in these regions by hydrothermal effect at 105 °C. At least, this increase in the cellulose II crystal sizes can take place only in the disordered regions of M-regenerated celluloses, which have undergone the swelling treatment with 20% NaOH. Thus, LODP values are not necessarily reflected by the original cellulose II crystal sizes along the chain direction especially for M-regenerated celluloses.

Moreover, as shown in Table 2, the peaks or shoulders approximately corresponding to DP 20 are present in all SEC elution patterns of the acid hydrolyzed products of M-native, regenerated and M-regenerated celluloses. These DP 20 peaks indicate the presence of cellulose II crystallites corresponding to DP 20 to some extents in all the cellulose II samples (Isogai et al. 2008). However, no peaks or shoulders corresponding to DP 20 were present in the SEC elution patterns of CUAs prepared from any cellulose II sample, as shown in Fig. 3 and Table 2. The cellulose II crystallites corresponding to DP 20 even present in the cellulose II samples may be too small in size to be degraded to further low molecular-mass compounds during TEMPO-mediated oxidation, and are removed from the isolated CUAs during purification process using aqueous 80% ethanol as the washing solvent. However, the CUA was quantitatively obtained from the regenerated cellulose II with DP 15 by the oxidation and the successive isolation and purification processes (Fig. 3 and Table 1). Thus, CUA fractions corresponding to DP 20 should be detected in the SEC elution patterns in Fig. 3 as peaks or shoulders, if these fractions are really present in M-native, regenerated and M-regenerated celluloses.

Thus, the cellulose II crystallites corresponding to DP 20 observed for the acid-hydrolyzed products of the cellulose II samples are likely not to be present in any original cellulose II samples (M-native, regenerated or M-regenerated cellulose) but are probably formed in the acid hydrolyzed products by depolymerization and some cellulose chain rearrangements in the disordered regions during the hydrolysis process at 105 °C. These results reveal again that some LODP values observed for acid-hydrolyzed products is not necessarily reflected by the crystal sizes along the cellulose chain direction present in the original crystalline celluloses but may form during the hydrolysis process at high temperatures.

Preparation of CUA from ball-milled native celluloses

Ball-milled celluloses were prepared from two native celluloses, cotton linters and CF11, and were subjected to the TEMPO-mediated oxidation. Both cotton linters and CF11 decreased in their crystallinity indices with the ball-milling time (Fig. 4); crystallinity indices of cotton linters after ball-milling for 0, 3, 6, 12 and 24 h were 0.36, 0.12, 0.09, 0.02 and 0.00, respectively, and those of CF11 were 0.40, 0.25, 0.14, 0.09 and 0.00, respectively. Thus, completely non-crystalline celluloses were prepared from cotton linters and CF11 by ball-milling of more than 12 and 24 h, respectively.

When these partly and completely non-crystalline celluloses prepared from native celluloses by ball-milling were oxidized in water at pH 10 by the

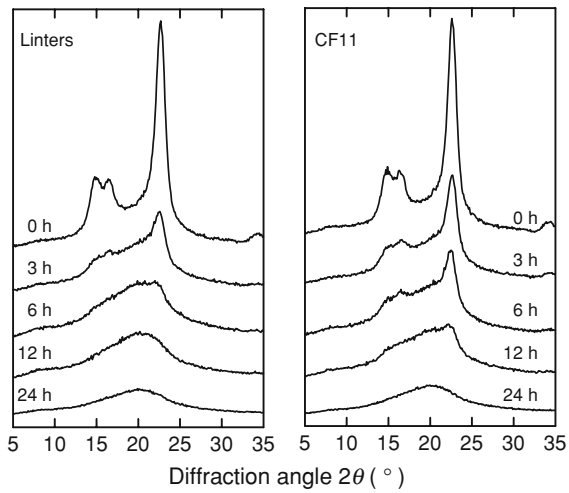

Fig. 4 X-ray diffraction patterns of cotton linters and Whatman CF11 ball-milled for various times

TEMPO-mediated system, some water-insoluble particles were present in the aqueous solutions after the oxidation. These water-insoluble fractions were removed by centrifugation, resulting in some decreases in the yields of the water-soluble oxidized products. As described later, ^{13}C-NMR analysis of the water-soluble fractions of the oxidized products revealed that the C6 primary hydroxyl groups of cellulose were completely oxidized to C6 carboxylate groups. Thus, CUAs can be prepared by the TEMPO-mediated oxidation of partly and completely disordered celluloses, which are prepared from native celluloses by ball-milling. Unlike the results of the cellulose II samples described in the previous section, the disordered regions, which account for the major portion of the ball-milled native celluloses, are partly depolymerized during the TEMPO-mediated oxidation but mostly converted to CUA molecules. Hence, in the case of the ball-milled native celluloses, the rate of the C6 oxidation is much higher than that of depolymerization on the whole.

SEC elution patterns of CUAs prepared from ball-milled native celluloses are shown in Fig. 5. All the CUAs prepared had similar and normal SEC elution patterns, and MM plots were mostly on the same line. The original DPv values of the ball-milled native celluloses and yields and DP values of CUAs prepared thereof by the TEMPO-mediated oxidation are listed in Table 3. When incompletely disordered celluloses were used, yields of CUAs were low because of the presence of significant amounts of water-insoluble particles in the aqueous solutions; only disordered regions in the ball-milled native celluloses can be converted to CUAs by the oxidation. Because ball-milling can be carried out to native celluloses without any chemicals or solvents, the preparation of CUAs from ball-milled native celluloses is somewhat advantageous, when compared with liquid ammonia-treated native celluloses, 20% NaOH-treated native celluloses or regenerated celluloses used as the starting materials in the TEMPO-mediated oxidation to prepare CUAs. Moreover, when the ball-milled native celluloses with DPv values of more than 110 are used as the starting materials, CUAs with DPw values of more than 70 can be obtained. These DPw values are clearly higher than those of CUAs prepared from regenerated or M-regenerated celluloses, although they are similar to those of CUAs prepared from M-native celluloses. Thus, CUAs with DP values similar to those prepared from M-native cellulose were obtained from the ball-milled native celluloses by the TEMPO-mediated oxidation. When the milling time was extended to 24 h, the DPv values of the ball-milled celluloses decreased to 45–63, and the DPw values of CUAs prepared thereof were only 28–32, which were lower than those of CUAs prepared from the cellulose II samples in Table 1.

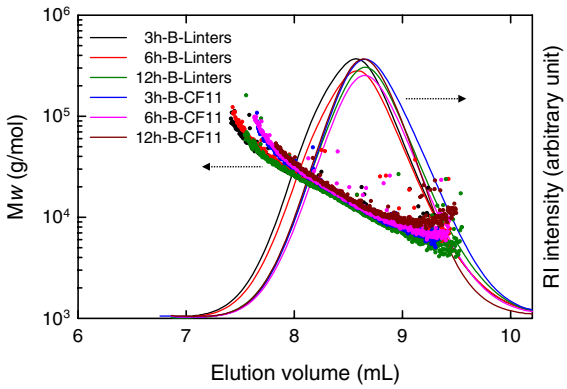

Fig. 5 SEC elution patterns and the corresponding molecular mass plots of cellouronic acids prepared from ball-milled cotton linters and Whatman CF11 by TEMPO-mediated oxidation

Table 3 Yield, DPw, DPn and DPw/DPn values of cellouronic acid Na salt (CUA) prepared from ball-milled cotton linters and Whatman CF11 by TEMPO-mediated oxidation

Milling time	DPv	CUA			
		Yield (%)	DPw	DPn	DPw/DPn
Cotton linters	800				
3 h	307	61	83	49	1.69
6 h	209	72	80	47	1.70
12 h	129	87	67	43	1.56
24 h	63	90	28	22	1.27
Whatman CF11	200				
3 h	189	43	72	45	1.60
6 h	164	56	76	46	1.65
12 h	113	77	82	60	1.37
24 h	45	82	32	21	1.52

Preparation of CUA from ball-milled wood saw dust

In this section, the saw dust of Japanese cedar was ball-milled to prepare a fine wood powder containing

non-crystalline cellulose, which was then subjected to the TEMPO-mediated oxidation. Figure 6 shows X-ray diffraction patterns of the wood saw dust before and after the ball-milling for 4 h. The cellulose I in the original wood saw dust was mostly converted to disordered cellulose by the ball-milling. After the oxidation for 2 h, the ball-milled wood saw dust was mostly dissolved in the aqueous medium, even though some water-insoluble particles were present in there. These water-insoluble particles were removed by centrifugation at 10,000*g* for 10 min, and the oxidized product was obtained and purified from the water-soluble fraction.

Figure 7 shows ^{13}C-NMR spectra of CUAs prepared from the 4 h-ball-milled wood saw dust, the 12 h-ball-milled cotton linters and the regenerated cellulose II with DP 15 by the TEMPO-mediated oxidation. Even though some small unknown signals were present at, for example, 70.7, 72.1, 100.7 and 186.6 ppm in the ^{13}C-NMR spectra of the CUA prepared from the ball-milled wood saw dust by the oxidation for 2 h, the obtained product was mostly regarded as CUA, which had no signal due to the C6 primary hydroxyl groups of cellulose around 61 ppm but had six main signals due to the glucuronic acid unit of CUA. Because no signals due to residual lignin were detected in the range from 110 ppm to 160 ppm, almost all lignin components in the ball-milled wood saw dust were degraded by the TEMPO-mediated oxidation, and removed from CUA molecules by the filtration and/or purification processes used. The extended oxidation time of the ball-milled wood saw dust to 10 h brought about some clear side-reactions, resulting in the formation of some additional unknown signals in the ^{13}C-NMR spectrum. The ^{13}C-NMR spectrum of the oxidized product prepared from the 12 h-milled cotton linters cellulose also had some unexpected signals especially at 94.5 and 96.8 ppm due to anomeric carbons of reducing ends formed by some side reactions as well as six main signals due to CUA molecules. The TEMPO-oxidized product prepared from the regenerated cellulose II with DP 15 had also the six main signals due to the glucuronic acid unit of CUA. The signal at 172.1 ppm may be due to C1 carboxylate groups formed from the C1 anomeric carbon of the reducing ends by the oxidation.

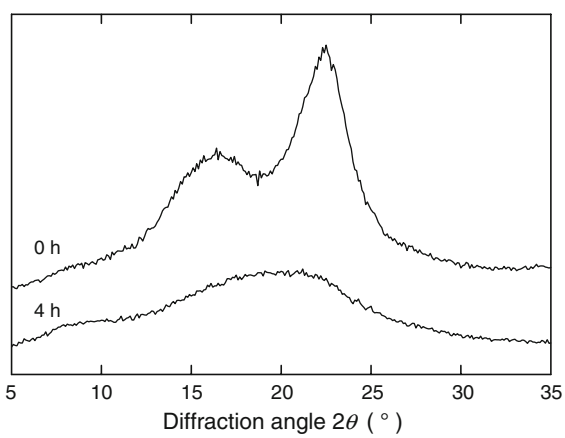

Fig. 6 X-ray diffraction patterns of the Japanese cedar saw dust before and after ball-milling for 4 h

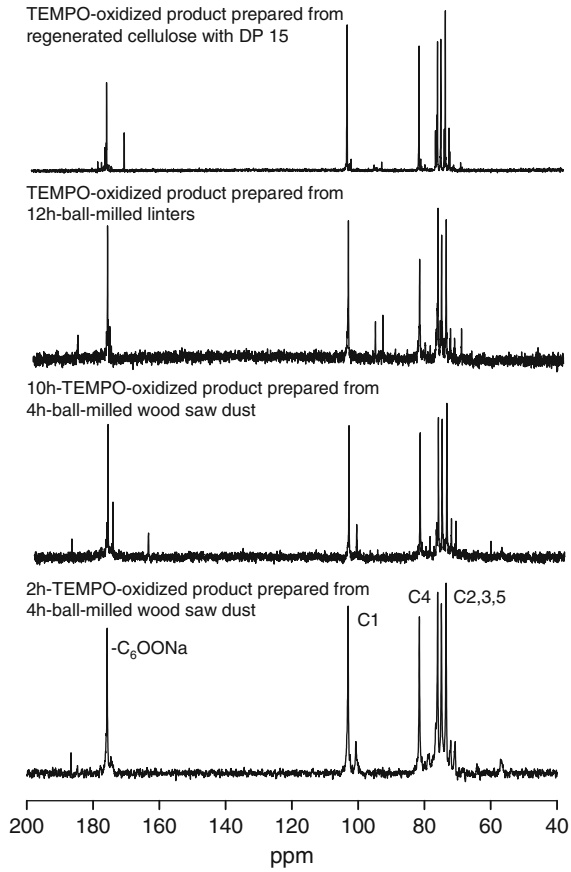

Fig. 7 ^{13}C-NMR spectra of TEMPO-oxidized products prepared from regenerated cellulose II with DP 15, 12 h-ball-milled cotton linters cellulose, ball-milled Japanese cider oxidized for 10 h and that for 2 h

The SEC elution patterns and the corresponding molecular mass plots of CUAs prepared from the ball-milled wood saw dust by TEMPO-mediated

oxidation for 2 h and 10 h are shown in Fig. 8. When the oxidation time was extended to 10 h, the SEC elution pattern of the oxidized product clearly shifted to higher elution volume or lower molecular-mass direction. Thus, the oxidation time is crucial to prepare CUAs with higher DP values. The MM plots of the CUA prepared by the oxidation for 10 h are slightly shifted to lower MM direction at the same elution volume, compared with that prepared by the oxidation for 2 h. Probably, CUA molecules with lower DP values may be present in the solution as more expanded conformations based on the Benoit–Doty theory (Benoit and Doty 1953; Yanagisawa and Isogai 2005).

The mass recovery ratio and DP values of CUAs prepared from the ball-milled wood saw dust by TEMPO-mediated oxidation for 2 and 10 h are listed in Table 4. When the oxidation time was 2 h, the mass recovery ratio of the oxidized product was 55% based on the weight of the starting ball-milled wood saw dust, which indicated that approximately 90% of cellulose in the ball-milled wood saw dust was obtained as CUAs by the TEMPO-mediated oxidation. Moreover, the DPw value of the CUA was about 170, which was clearly higher than those of CUAs prepared from other ball-milled cellulose samples or cellulose II samples. Thus, CUA with the highest DP value examined so far was quantitatively obtained from the 4 h-ball-milled wood saw dust by the TEMPO-mediated oxidation. This procedure to prepare CUA may be applicable to other ball-milled wood saw dust samples to provide CUAs with higher DP values.

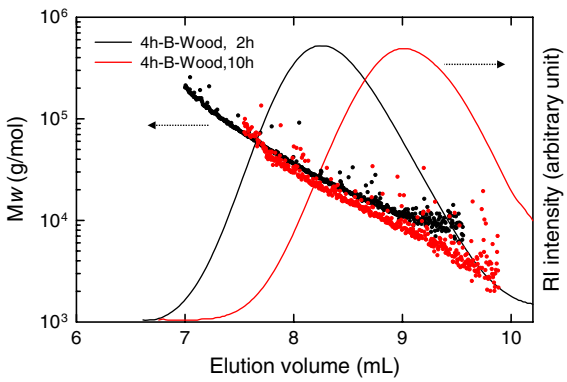

Fig. 8 SEC elution patterns and the corresponding molecular mass plots of cellouronic acids prepared from 4 h-ball-milled Japanese cider by TEMPO-mediated oxidation for 2 h and 10 h

Table 4 Mass-recovery ratios, DPw, DPn and DPw/DPn values of cellouronic acid Na salt (CUA) prepared from 4 h-ball-milled Japanese cedar by TEMPO-mediated oxidation

Oxidation time	Mass recovery ratio (%)	DPw	DPn	DPw/DPn
2 h	55	168	64	2.63
10 h	–	56	24	2.33

Conclusions

When M-native, regenerated and M-regenerated celluloses were used as the starting materials of the TEMPO-mediated oxidation, CUAs were obtained in the yields of 84–94%. However, the DPw values are in the range of 38–79, and thus significant depolymerization of cellulose chains is inevitable. These DPw values of CUAs obtained are probably reflected by the cellulose II crystal sizes along the chain direction in the original cellulose II samples; the disordered regions in the cellulose II samples are first depolymerized to form microcrystals, and then water-soluble CUA molecules are formed and peeled off from the microcrystal surfaces by TEMPO-mediated oxidation. In some cases, the DPw values of the CUAs prepared were inconsistent with LODP values obtained by dilute acid hydrolysis of the same cellulose II samples at high temperatures. Thus, the LODP values are not necessarily reflected by the cellulose crystal sizes along the chain direction in the original celluloses, but are formed in the disordered regions during acid hydrolysis at high temperatures by some molecular rearrangements. CUAs can be obtained in good yields also by TEMPO-mediated oxidation of native celluloses, when their crystalline cellulose I structures are mostly converted to disordered ones by ball-milling. However, the DPw values of CUAs prepared from the ball-milled native celluloses were lower than about 80. Hence, although the rate of the C6 oxidation of the ball-milled native celluloses is higher than that of depolymerization in TEMPO-mediated oxidation, the partial decreases in DP of CUAs are still inevitable. On the other hand, CUA with DPw of about 170 was obtained from ball-milled wood saw dust by TEMPO-mediated oxidation, which is a promising procedure to prepare CUAs with higher DP values.

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