Biomacromolecules

June 2006 Published by the American Chemical Society Volume 7, Number 6

Communications

Homogeneous Suspensions of Individualized Microfibrils from TEMPO-Catalyzed Oxidation of Native Cellulose

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Received February 20, 2006; Revised Manuscript Received April 15, 2006

Never-dried native celluloses (bleached sulfite wood pulp, cotton, tunicin, and bacterial cellulose) were disintegrated into individual microfibrils after oxidation mediated by the 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) radical followed by a homogenizing mechanical treatment. When oxidized with 3.6 mmol of NaClO per gram of cellulose, almost the totality of sulfite wood pulp and cotton were readily disintegrated into long individual microfibrils by a treatment with a Waring Blendor, yielding transparent and highly viscous suspensions. When observed by transmission electron microscopy, the wood pulp and cotton microfibrils exhibited a regular width of 3−5 nm. Tunicin and bacterial cellulose could be disintegrated by sonication. A bulk degree of oxidation of about 0.2 per one anhydroglucose unit of cellulose was necessary for a smooth disintegration of sulfite wood pulp, whereas only small amounts of independent microfibrils were obtained at lower oxidation levels. This limiting degree of oxidation decreased in the following order: sulfite wood pulp > cotton > bacterial cellulose, tunicin.

Introduction

Cellulose is produced in nature as slender micron-sized microfibrils having diameters ranging from 2 to 20 nm that depend on their biological origin.[1] The production of these elements is well regulated as they are continuously synthesized by an enzymatic machinery that not only assembles cellulose molecules but also spins them concomitantly through well calibrated biological spinnerets.[2] From this unique mode of biogenesis, the result is that the microfibrils are made of perfectly aligned cellulose molecules well organized into a nearly defect-free crystalline arrangement. The perfection of this organization leads to outstanding mechanical properties,[3] and for this reason, the extraction and use of individual cellulose microfibrils present a great potentiality for a number of applications, ranging from fluids with specific rheological properties[4] to the processing of nanocomposites.[5,6] As precursors of these products, homogeneous dispersions of individual microfibrils in aqueous or organic solvents are required.

The main source of cellulose microfibrils is found in plant cell walls such as those of wood or cotton fibers. In these, the microfibrils are so tightly hooked to one another by multiple hydrogen bonds that their extraction has proven extremely difficult. By using harsh aqueous mechanical treatment, it is possible to disintegrate pulp fibers from wood into suspensions of fibrils to obtain the so-called microfibrillated cellulose (MFC),[7,8] commercialized for industrial applications in the fields of food industry or that of filtration. Other systems rely on the mechanical disruption of primary wall cellulose where the microfibrils are less tightly wound than in the more common secondary walls.[9,10] Despite the high energy required by these treatments, the resulting products consist mainly of bundles of microfibrils and so far, it has not been possible to individualize cellulose microfibrils using solely mechanical disruption.

Another more promising route toward the preparation of suspensions of individual cellulose microfibrils relies on chemical treatments involving the addition of negatively charged

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10.1021/bm060154s CCC: $33.50
Published on Web 05/03/2006

entities at the microfibril surface. These charges and their repulsive effect greatly enhance the ease of separation of individual microfibrils. Classically, this is achieved by treating cellulose with sulfuric acid to obtain stable aqueous dispersions of cellulose microcrystals that are surface charged by sulfate groups.[11,12] However, this treatment is hydrolytic and yields a dramatic decrease in the microfibril length down to about 100−200 nm. Following another approach, Lepoutre et al.[13] have grafted anionic polyelectrolyte on never dried wood-pulp fibers. When the amount of grafted polymer exceeded 40% of the cellulose weight, the fibers could remarkably be disintegrated into individual microfibrils with a simple homogenizing treatment, using a Waring Blendor.[4]

Recently, the 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) radical, which was found to catalyze the oxidation of primary alcohol groups in aqueous media,[14] was applied with success to various cellulose products.[15−22] With this oxidative reaction, it was found that regenerated cellulose could be completely converted into water-soluble polyglucuronic acid.[17,18] In the case of native cellulose fibers, the oxidation proceeded throughout the fibers but occurred only at the surface of the microfibrils, which therefore became negatively charged.[20] Despite this beneficial surface derivatization, it has not been so far possible to disintegrate the TEMPO oxidized cellulose fibers into individual microfibrils, probably because the oxidation was achieved on once-dried fibers that are renown to irreversibly loose some of their accessibility and reactivity during their first drying cycle. In the present report, we show that the use of never-dried cellulose overcomes this problem and that, following a controlled TEMPO mediated oxidation, these samples can easily be disintegrated into individual microfibrils by a simple mechanical treatment. This protocol was applied with success to samples from four different sources of cellulose.

Experimental Section

Materials. A sample of never-dried cotton in a closed boll, corresponding to a 4 weeks post-anthesis, was kindly provided by Dr. Alfred French. Never-dried bleached sulfite pulp from *Pinus pinaster* with 30% solid content was shipped from Tembec Tartas pulping factory and stored at 4 °C before use. Part of the sulfite wood pulp was dried in an oven at 60 °C for 2 days before use as a control. The α-cellulose content of the pulp was about 92−94%. *Acetobacter xylinum* ATCC 12733 was cultivated in a protein-enriched glucose medium for 3 weeks under static conditions to produce cellulose pellicles that were then purified with a 0.5% sodium hydroxide solution. Tests of *Halocynthia roretzi* were purified using a standard method consisting of alternating treatments with alkali and acetate-buffered sodium chlorite.[23] TEMPO, sodium bromide, sodium hypochlorite solution, and other chemicals were of laboratory grade and used without further purification.

TEMPO-Mediated Oxidation of Cellulose. The never-dried cellulose (2 g of cellulose content) was suspended in water (150 mL) containing TEMPO (0.025 g) and sodium bromide (0.25 g). The TEMPO-mediated oxidation of the cellulose slurry was started by adding various amounts of 13% NaClO and conducted at room temperature under gentle agitation.[15] The pH was maintained at 10.5 by adding 0.5 M NaOH. When no more decrease in pH was observed, we considered that the reaction was finished and adjusted the pH to 7 by adding 0.5 M HCl. The TEMPO-oxidized product was thoroughly washed with water by filtration and stored at 4 °C before further treatment or analysis. The carboxylate content in the oxidized solid product was determined using an electric conductivity titration method.[21] Aliquots of each sample were further oxidized with $NaClO_2$ at pH 4−5, and the increase in carboxyl groups was regarded as the amount of aldehyde groups present in the TEMPO-oxidized celluloses.[21]

Mechanical Disintegration. For the sulfite wood pulp, slurries with different solid contents were homogenized with a Waring Blendor. For samples in small quantity, an inox microvolume bowl was used. The viscosity rose progressively until the blade started to rotate in air. Each time the viscosity was too high, distilled water was then added to disintegrate further. The suspension thus obtained was centrifuged at 12 000*g* to separate large particles from the microfibrils. For tunicin and bacterial cellulose, the sample was sonicated for a few seconds until the suspension temperature increased by about 5 °C.

Turbidimetry. Cellulose suspensions were introduced into standard disposable cuvettes, and the transmittance was measured between 300 and 1100 nm using a Cary50 BIO UV−vis spectrometer. The spectrum of a cuvette filled with water was measured as blank and was used to correct the transmittance of the samples.

Fourier Transform Infrared Spectrometry (FT-IR). Thin films were cast from the suspensions on a Teflon plate. FT-IR spectra were recorded on these films using a Perkin-Elmer 1720X in transmission mode with a resolution of 4 cm^{-1}. The transmittance was converted into absorbance for display.

Transmission Electron Microscopy (TEM). Drops of dilute microfibrils suspensions were deposited onto glow-discharged carbon-coated electron microscopy grids. The excess liquid was absorbed by a piece of filter paper, and a drop of 2% uranyl acetate negative stain was added before drying. The liquid in excess was blotted, and the remaining film of stain was allowed to dry. The specimens were observed using a Philips CM200 microscope operating at 80 kV. Micrographs were recorded on Kodak SO163 films.

Results and Discussion

Table 1 summarizes the characteristics of the samples. Under our conditions, the TEMPO-mediated oxidation did not affect the morphology of any sample at macroscopic level, as has been reported earlier.[21,22] No significant swelling was observed compared to the initial cellulose. The oxidation as monitored by NaOH consumption was similar for never-dried and dried pulps, proceeding very rapidly up to the oxidation of about 20% of the anhydroglucose unit and ending in about 10 min, almost quantitatively generating aldehyde and carboxylate groups. For cellulose samples containing larger microfibrils, the limit of this rapid oxidation was lower. Then, oxidation took much longer for the following 3%.

When the slurries of sulfite wood pulp were homogenized with a Waring Blendor, a significant increase in viscosity was observed if the solid content was between 2 and 4% (w/v). On the other hand, such increase was absent with concentrations of 1% and lower. The increase in viscosity in the more concentrated slurry was quite fast if the oxidation had been achieved with 3.8 mmol of NaClO per gram of cellulose but occurred at a slower rate if the oxidation had been performed with 2.5 mmol/g cellulose. If less NaClO had been used, no gel could be obtained and only a slight increase in viscosity could be reached. When the slurries had reached a gel state, the blade of the Waring Blendor turned in the air. A stepwise addition of water in the homogenizer allowed a further mechanical disintegration of the fiber, until the concentration of 0.5% solid was reached.

When fragments of fibers remained in the suspension, transparent suspensions could be obtained by removing the fragments by centrifugation, typically at 12 000*g* after diluting the solid content to 0.1−0.15% to decrease the viscosity. The fragments of fibers, thus collected as sediments, could thus be further treated. In fact during the mixing, due to the high viscosity, a certain amount of the sample stayed on the wall of the Waring Blendor and did not effectively undergo mechanical impact. By repeating such treatments on sulfite pulp oxidized with 2.5 or 3.8 mmol $NaClO/g_{cellulose}$, more than 90% of the material went into the supernatant fraction. At a lower oxidation

Table 1. Characteristics of TEMPO-oxidized Cellulose Samples

sample	NaClO added [mmol/g]	reaction time [min]	solid recovery [%]	COONa content [mmol/g] (DO[a])	CHO content [mmol/g] (DO[a])	total DO[a]	efficiency[b] [%]	yield[c] 1st treatment [%]	yield[c] 3rd treatment [%]
never-dried sulfite pulp	0		100	0.04 (0.01)	0.00 (0.00)	0.01			
	1.3	5	97	0.45 (0.07)	0.35 (0.06)	0.13	95	2	5
	2.5	11	98	0.99 (0.16)	0.33 (0.05)	0.21	91	52	91
	3.8	42	95	1.23 (0.20)	0.25 (0.04)	0.24	70	61	98
dried sulfite pulp	2.5	14	96	0.92 (0.15)	0.29 (0.05)	0.20	84	17	27
	3.8	46	96	1.30 (0.21)	0.23 (0.04)	0.25	73	30	52
	6.3	120[d]	93	1.52 (0.25)	0.23 (0.04)	0.28	51	60	99
never-dried tunicin	0		100	0.03 (0.00)	0.00 (0.00)	0.00			
	0.5	28	94	0.21 (0.03)	0.10 (0.02)	0.05	98	4	
	0.9	94	96	0.28 (0.05)	0.05 (0.01)	0.05	58	29	
	1.8	120[d]	90	0.31 (0.05)	0.03 (0.00)	0.06	33	71	
never-dried bacterial cellulose	1.8	12						98	
never-dried cotton	1.8	25						20	
	3.6	120[d]							96

[a] Degree of oxidation per anhydroglucose unit of cellulose. [b] Efficiency is defined by $(C_{CHO} + 2C_{COOH})/C_{NaClO}$, where C_{CHO} and C_{COOH} are the molar amount of aldehyde and carboxyle groups generated and C_{NaClO} is the amount of NaClO added. [c] Weight of microfibrils after removal of centrifugation sediments. [d] The reaction was stopped after 120 min by an addition of ethanol.

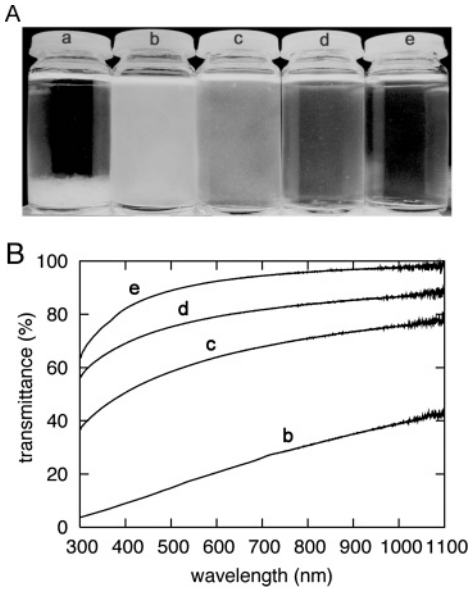

Figure 1. (A) Photograph of 0.1% bleached sulfite wood pulp suspensions treated with a Waring Blendor after TEMPO-mediated oxidation with different amounts of NaClO per gram of cellulose: (a) 0, (b) 1.3, (c) 2.5, and (d) 3.8 mmol. (e) Supernatant of suspension c after centrifugation at 12 000*g* for 30 min. (B) UV−vis transmittance spectra of the same suspensions with a 1 cm path length.

level, resistant particles that could hardly be disintegrated remained in higher quantity.

Figure 1A shows the appearance of disintegrated sulfite wood pulp cellulose suspensions at 0.1% with different degrees of oxidation (Figure 1Aa−d), as well as the transparent supernatant after centrifugation (Figure 1Ae). The UV−vis transmittance spectra of the same suspensions are presented in Figure 1B. The transmittance is wavelength-dependent and is lower at shorter wavelengths, as light scatters more when the wavelength approaches the diameter of the particles. For suspended fibrils or rods thinner than the wavelength, the light scattering is proportional to the mass/length ratio or the cross section area.[24] This explains why, as the disintegration proceeds, the suspension becomes more and more transparent at the same solid concentration.

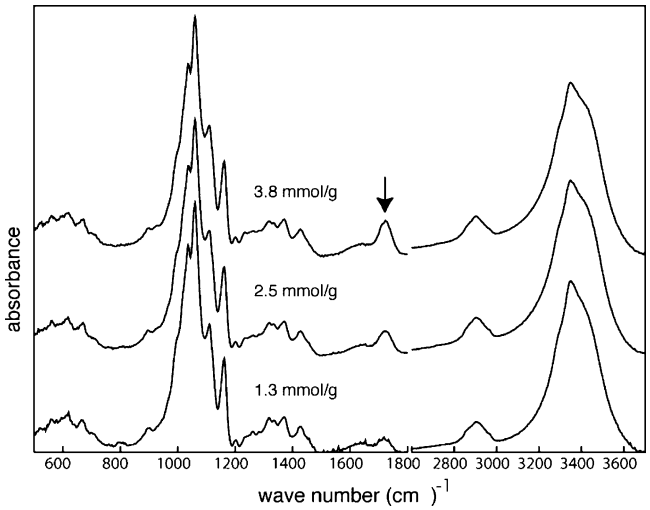

Figure 2. FT-IR spectra of cellulose microfibrils TEMPO-oxidized with different amounts of NaClO per gram of cellulose: (a) 1.3, (b) 2.5, and (c) 3.8 mmol. Arrow indicates the carbonyl band.

Figure 3. Transparent suspension of 0.1% cellulose microfibrils of never-dried sulfite wood pulp TEMPO-oxidized with 2.5 mmol NaClO per gram of cellulose. Observed at rest between crossed polarizers.

The homogenization treatment had less impact on highly crystalline cellulose samples (tunicin and bacterial cellulose), which were more efficiently disintegrated by sonication. Bacte-

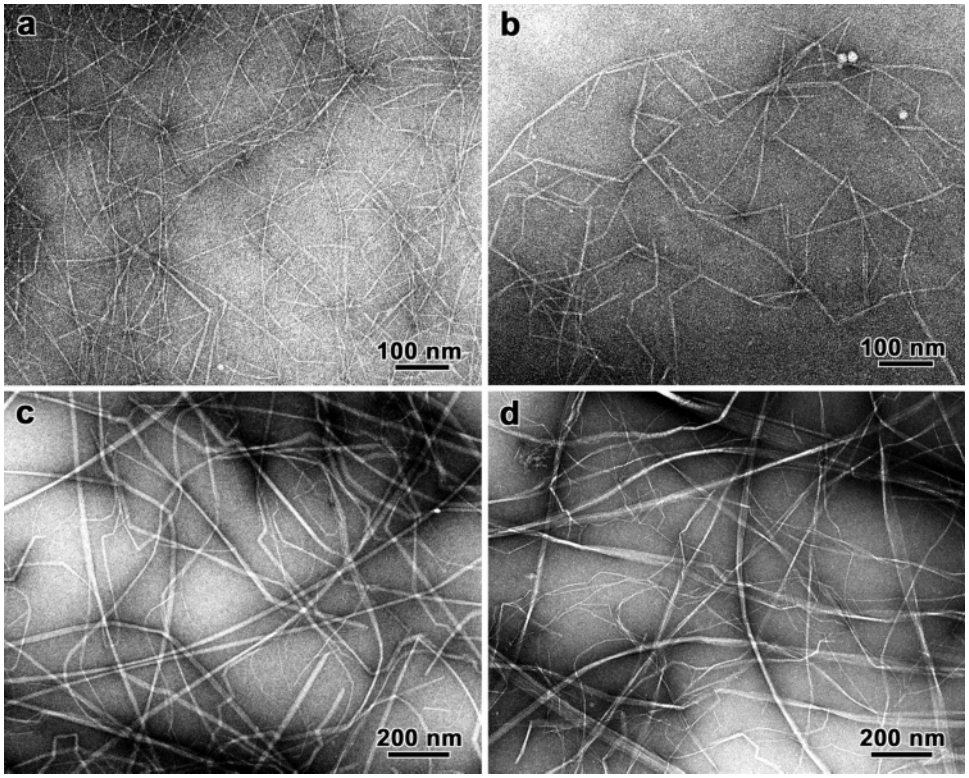

Figure 4. Transmission electron micrographs of cellulose microfibrils disintegrated after TEMPO-mediated oxidation of never-dried samples: (a) bleached sulfite wood pulp, (b) cotton, (c) tunicin, and (d) bacterial cellulose. The preparations were negatively stained with uranyl acetate.

rial cellulose readily dispersed to form translucent suspensions using sonication only. Tunicin dispersed likewise when sonication was used after the mechanical treatment. The particles that were not completely disintegrated into microfibrils were separated as sediments by centrifugation at 6000*g*. The type of mechanical treatment that is efficient to disrupt the association of microfibrils depends on the organization of microfibrils in the parent tissue. However, without oxidation, the microfibrils did not suspend in water after mechanical treatment only. Thus, a TEMPO-mediated oxidation clearly helps the disintegration process, probably by loosening the adhesion between microfibrils and electrostatic repulsions between microfibrils, where significant amounts of carboxylate groups were introduced. In fact, a significant increase in water retention value has been observed for cotton oxidized to carboxylate contents above 0.7 mmol/g.[21]

Depending on the oxidation level, different percentages of sulfite pulp cellulose are susceptible to microfibrillation. To clarify whether only microfibrils that were oxidized to saturation went to the supernatant, we recorded infrared spectra of the supernatant obtained at different oxidation levels (Figure 2). At higher oxidation levels, the absorbance due to carbonyl groups at 1738 cm^{-1} increased, indicating that the reaction occurred rather homogeneously throughout the fiber and that the disintegration did not require the complete oxidation of the surface. The supernatant thus obtained was transparent and showed a frozen-in birefringence at rest when observed between crossed polarizers (Figure 3).

Figure 4 shows TEM images of oxidized cellulose microfibrils from the transparent supernatant. The microfibrils are well individualized, but isolated microfibrils could rarely be observed even after extensive dilution, in which case domains with a significant microfibril concentration were observed on the carbon film. Kinks were observed on microfibrils in all samples probably due to damage from the mechanical treatment. The lateral size of microfibrils from sulfite wood pulp was on the order of a few nanometers, whereas that of cotton was about twice as large. Tunicin cellulose exhibited two classes of microfibrils, one with a width of about 10−20 nm and the other with a lateral size comparable to that of cotton microfibrils. These two classes of widths were probably present in the parent tissue as can be seen in TEM images of thin cross sections of tunicate mantle.[25] Bacterial cellulose also exhibited two classes of microfibrils, one consisting of 50−100-nm-wide ribbons with occasional internal cracks and the other comparable to cotton microfibrils. In all cases, we did not observe the lateral aggregation that is often seen in acid-hydrolyzed samples,[11,12] and the lateral dimension of the microfibrils measured from the TEM images almost corresponded to the lateral crystallite size in the parent sample as measured by wide- and small-angle X-ray scattering techniques.[26,27] The small width makes the suspension transparent and significantly viscous even at very low solid fraction.

Compared to the conventional technique to disintegrate cellulose fibers into microfibrillar suspensions using mechanical treatment only, a combination of TEMPO-mediated oxidation and homogenizing treatment presents the advantage to disintegrate into microfibrils with smaller widths, using a much lower energy input.

Acknowledgment. We thank M. F. Marais (CERMAV) for assistance in culture and purification of bacterial cellulose, as well as Tembec Tartas and Dr. Alfred French of the USDA for the gift of bleached sulfite pulp and cotton samples, respectively. We are grateful to H. Chanzy for helpful suggestions and a critical reading of the manuscript. This research project has been supported by a Marie Curie Early Stage Research Training Fellowship of the European Community's Sixth Framework Program under Contract No. MEST-CT-2004-503322.

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BM060154S