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Swelling of *Valonia* Cellulose Microfibrils in Amine Oxide Systems

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Swelling of Crystalline Cellulose Microfibrils in Amine Oxide Systems

by
Pierre Noé and H. Chanzy

ABSTRACT

Cellulose microfibrils from *Valonia ventricosa* cell-wall fragments were immersed into molten N-methylmorpholine-N-oxide monohydrate (NMMO. H₂O), stabilized with *n*-propyl gallate and kept at 80°C. The resulting ultrastructural modifications, which were followed by transmission electron microscopy and electron diffraction analysis, showed that within minutes, the solvent slowly penetrated inside the crystalline microfibrils and progressed as a wedge in between the cellulose chains without cutting them. Prior to dissolution, a longitudinal sub-fibrillation of the initial microfibrils resulted, leading to the observation of highly swollen microfibrils, which could reach diameters up to three times larger than those of the initial samples. This mode of swelling is compared with those occurring in other systems, where the intra-crystalline swelling of cellulose has been described at the ultrastructural level.

KEYWORDS: Cellulose swelling; *Valonia* cellulose; N-methylmorpholine-N-oxide.

RESUME

Des microfibrilles en provenance de fragments de paroi de *Valonia ventricosa* ont été immergées à 80°C dans le N-oxyde de N-méthylmorpholine monohydraté (NMMO. H₂O), en présence de gallate de *n*-propyle. La modification de l'ultrastructure de ces microfibrilles a été étudiée par microscopie électronique à transmission et analysée par diffraction des électrons. On observe un gonflement de ces microfibrilles sous l'influence du solvant qui pénètre comme un coin à l'intérieur de celles-ci, en se glissant entre les chaînes cellulosiques. Dans l'étape ultime, avant la dissolution, on remarque des microfibrilles ayant gonflé jusqu'à un diamètre trois fois supérieur au diamètre initial. Ce mode de gonflement est comparé avec d'autres systèmes de gonflement intra-cristallins de la cellulose.

Introduction

Cellulose can be considered as the archetype of a renewable resource susceptible to yield structural materials of high mechanical properties. For this reason and besides the uses of native cellulose fibers in pulp and paper commodities, the processing of cellulose into various other products is the focus of a never-ending interest. Developed more than a century ago (1), the viscose process has traditionally been the leading method to yield cellulose products, such as yarns, fibers, films, cellular products, etc. Today, however, the viscose process is slowly being abandoned, since it presents serious environmental drawbacks. As a consequence, alternative ways of processing cellulose by finding environment friendly new solvent systems have been, and still are, the focus of intense research and development.

Among the new cellulose solvents that have been reported, N-methylmorpholine-N-oxide (NMMO) stands out as being the only organic solvent that has successfully reached the stage of commercialization (2), leading to the development of new cellulose fibers under the trade names of Tencel and Lyocell. Today, these fibers are being successfully incorporated in high-class clothing, non-woven fabrics as well as in a number of specialty products. As opposed to the viscose process, the dissolution of cellulose in NMMO does not require any derivatization. In this system, the dissolution and regeneration process is derived from the hydrogen-bonding characteristics of the hygroscopic NMMO, which will interact with cellulose if there is a shortage of water, but will reject cellulose if water is in excess. As the melting point of NMMO is inversely related to its water content, the dissolution of cellulose can thus be monitored by temperature and water content. Typical phase diagrams (3, 4) have shown that NMMO monohydrate (NMMO. H₂O: 13.3 % water w/w) acted as a solvent at temperatures in the order of 100°C, whereas NMMO disquihydrate (NMMO.2.5 H₂O: 28%water w/w) did not lead to solubilization at any temperature.

Observations of the dissolution of various cellulose fibers into NMMO have shown that if NMMO monohydrate was used at temperatures of 80°C and above, ramie fibers readily dissolved by breaking down without appreciable swelling (5). On the other hand, if 18-20 % w/w water was present in the system, extensive swelling occurred as the diameter of the fibers could increase several folds without any dissolution. Such behavior was also reported for other fibers such as those of cotton or wood pulp, but the swelling occurred in the form of ballooning (5), thus revealing the inner organization of the cellulose microfibrils within the cell walls of these fibers. With ramie as well as with the other fibers, the swelling process took a substantial time to reach its limit, in contrast with their dissolution, which occurred readily when the water concentration conditions were sufficiently low. At the cellulose crystal level, X-ray and ^{13}C NMR studies have shown that in the case of swelling, the NMMO molecules were able to pry open the $(1\bar{1}0)$ crystallographic planes while the dense equatorial planes such as the (110) and (200) were less sensitive to the solvent influence (5-7). On the other hand, no crystalline NMMO-cellulose complex could be detected.

Whereas X-ray data give only global information, the ultrastructural details of the swelling of the cellulose crystal under the thrust of hydrated NMMO molecules is not known. This work was therefore undertaken to answer this question. For this, we have used *Valonia* cellulose microfibrils and followed their swelling by transmission electron microscopy, together with selected area electron diffraction analysis. Individual *Valonia* microfibrils, which are easily disrupted from *Valonia* cell walls, have such large dimensions, that they can be readily observed in their finer details by transmission electron microscopy (8, 9) and analyzed locally by electron diffraction (10, 11), diffraction contrast imaging (12, 13) or high resolution AFM (14, 15). In view of their high crystallinity and perfection, they have become ideal objects to model any solid-state interaction of cellulose with chemical (16, 17) or enzymatic reagents (18, 19). Indeed, these transformations can be unambiguously followed,

both in image and diffraction modes, with a transmission electron microscope. It is believed that the observations made on the transformation of *Valonia* cellulose can be transposed to narrower and less organized cellulose, where the microfibrils are too small to be investigated individually by electron microscopy.

Experimental

Chemicals

N-Methylmorpholine N-oxide (NMMO) and *n*-propyl gallate were purchased from Aldrich. NMMO was recrystallized in dry acetone in the form of NMMO. H₂O (melting point 80°C).

Valonia cellulose

Valonia ventricosa single-cell vesicles were harvested in the Lower Keys, Florida. After puncturing the cells, their walls were dried by pressing on tissue paper. The dried cell walls were immersed overnight in 0.5% (w/w) aqueous HCl to remove the incrusting calcite and then, after adequate washing, were boiled for 2 hours in 2% aqueous NaOH solution. After rinsing thoroughly with water, wall fragments were delaminated with a pair of tweezers under a stereomicroscope. The resulting fragments with individualized microfibrils were used as such for subsequent swelling experiments.

Swelling and dissolution

The swelling was achieved by immersing the *Valonia* cell wall fragments into solutions of NMMO. H₂O containing 2% *n*-propyl gallate and kept at 80°C for times ranging from a few minutes to four hours. The swollen fragments were removed and washed thoroughly with methanol. For the samples, which had dissolved, the corresponding solutions were diluted with DMSO, cooled and then dialyzed against water to yield a flocculent precipitate.

Transmission electron microscopy (TEM)

The swollen samples as well as the precipitate resulting from dissolution were deposited on carbon-coated grids. Some samples were negatively stained with uranyl acetate for imaging

purpose whereas others, which were investigated by electron diffraction analysis, were kept unstained. Observations were achieved with a Philips EM 400 T electron microscope operated at 80 kV and normal illumination for imaging purpose and 120 kV and reduced electron beam for electron diffraction experiments. Calibration of the diffraction diagrams was achieved by depositing some specimens on gold sputtered carbon coated grids and comparing the diameters of the gold diffraction rings with those of the diffraction spots of the specimens.

Results and discussion

Confirming known descriptions (9), the initial *Valonia* cellulose fragments that were used here, consist of arrays of slender featureless microfibrils, having rigorously parallel edges and lengths of many microns. Whereas each microfibril has a constant diameter throughout its length, there is a variation of these diameters, which range from 25 nm for the largest to no more than 15 nm for the narrowest. This distribution is well illustrated in Figure 1a, where out of the three microfibrils, two have a diameter of 24 nm whereas the third one is only 18 nm wide. When the *Valonia* fragments were immersed in molten NMMO. H₂O at 80°C, a slow swelling was observed before total dissolution. Figures 1b-1d correspond to local images depicting the various aspects of a fragment that was immersed for 10 minutes. These figures illustrate the heterogeneity of the swelling action of NMMO on these highly crystalline microfibrils: in the same sample some microfibrils were barely affected, whereas others were extensively swollen and perhaps some even dissolved. In Figure 1b, the swelling, which is initiated in the middle of a microfibril, produces an initial delamination and from there, the progression of the solvent is prying open the intact part of the microfibril in both directions. Interestingly, the swelling does not lead to any sectioning of the microfibril, but exclusively produces a longitudinal sub-fibrillation into no more than three or four elements, the thicker one being about 7 nm wide. These sub-fibrils, which are parallel to one another, are seen without interruption along the whole swollen area. In Figure 1c, a bundle of 4

microfibrils is being swollen in unison, starting from the right hand side of the micrograph toward the left. Again, the delamination into sub-fibrils is clearly visible, and as in Figure 1b, the progression of NMMO into the microfibril can be envisaged as resulting from a solvent wedge, slowly progressing in the core of the microfibril. The result is a delamination of the

Figure 1

microfibril into narrower elements, but again without apparent breakage into shorter segments. In Figure 1d, three completely swollen microfibrils are represented. The widest one has a diameter of 60 nm, i.e about three times the initial diameter whereas the other two are respectively 48 and 44 nm wide. Each of these swollen microfibrils consists now of a bundle of about a dozen of undulating sub-fibrils more or less organized parallel to the microfibril direction, but having no more than 2 to 3 nm in diameter. Whereas the microfibril located at the top of Figure 1d shows some trace of dissolution with loosened fibrils tips in its upper border, the other two are only swollen and do not present any hint of dissolution or transversal fracturing.

Figure 2

In Figure 2, the state of swelling and its concomitant decrystallization is followed by electron diffraction analysis. Figure 2a is typical of a fiber diffraction diagram of a bundle of initial *Valonia* microfibrils oriented with vertical axis. A pattern such as this one contains several hundred diffraction spots extending to a resolution better than 0.1 nm in the fiber direction and 0.2 nm in the equatorial direction. Such wealth of data, which is typical of *Valonia* cellulose (10), denotes a high coherence of large crystalline domains, which encompass the whole width of the crystalline microfibrils. Figure 2b corresponds to a bundle of parallel swollen microfibrils, where the sub-fibrillation is of the type shown in Figure 1b, with elements having of the order of 7 nm in width. In this pattern, one still recognizes the main features of the fiber diffraction diagram of native cellulose (cellulose I), but the

resolution is much lower than that of the initial pattern. In fact, only the strongest diffraction spots are clearly visible on the equator, the meridian and the second layer line. This pattern is very similar to a classical electron diffraction diagram of cotton (20), i.e. that of cellulose made of native crystalline microfibrils having of the order of 5 to 6 nm in diameter (21). The diagram in Figure 2c corresponds to a sample such as the one shown in Figure 1d. This diagram consists of only two arced equatorial diffraction spots with d -spacings centered at 0.4 and 0.56 nm together with four arced spots on the second layer line, calibrated at 0.43 nm. These features indicate that one is still dealing with native cellulose, but with samples of low crystallinity. In fact, the diagram in Figure 2c is very similar to that of primary wall cellulose, where the individual microfibrils are less than 3 nm wide and one cannot decide whether they belong to the cellulose I or cellulose IV₁ crystalline systems (22). Another aspect of the diagram in Figure 2c is that unlike samples of primary wall cellulose, it does not present any meridional diffraction spot. This absence reveals that the longitudinal coherence of the crystalline domains along the microfibrils is rather poor, in agreement with the undulating aspect of the sub-fibrils in Figure 1d. Figure 2d is the diagram of a sample that was left for 4 hours into NMMO. H₂O at 80°C, followed by re-precipitation. Under these conditions, the sample that had been totally dissolved and re-precipitated, does not show any hint of left over microfibrils. The diagram, which consists of only one ring at a d -spacing of 0.4 nm, can be categorized as belonging to the cellulose II allomorph, but with a rather poor crystallinity.

Figure 3

The results presented in this study are informative since they give ultrastructural details on the progression of NMMO into a crystalline cellulose microfibril, prior to its dissolution. The images shown in Figure 1 indicate that once the amine-oxide has started to swell a given microfibril, the solvent wedges its way along the microfibril by cutting the hydrogen bonds that hold the cellulose chain together. This mechanism is schematically

illustrated in Figure 3, which sketches the penetration of NMMO. H₂O into a typical crystalline *Valonia* microfibril consisting of ~1 000 chains. In this sketch, which would correspond to the situations in Figures 1b of 1c, the initial microfibril is split into 13 fibrous blocks, each having from ~ 40 to 100 chains and lateral dimensions ranging from 60 to 110 nm. In addition, the blocks have been drawn with a somewhat narrower dimension along the (110) planes, but wider along the (1 $\bar{1}$ 0) planes of the lattice, in agreement with earlier description (5). Obviously, a subsequent penetration of the solvent will divide further these initial blocks, which will thus become narrower and narrower until individual chains are surrounded by the solvent, with the result of total dissolution. Thus, a given microfibril subjected to a solution of NMMO. H₂O at 80°C will successively loose its "crystalline *Valonia*" characteristic denoted by its well-resolved electron diffraction diagram (Figure 2a) to adopt a cotton-like character (Figures 1b and 2b) and then that of a primary-wall cellulose sample (Figures 1d and 2c), before being completely dissolved.

Unlike in the case of ramie fibers, which dissolve readily into NMMO. H₂O, it seems that the highly crystalline *Valonia* microfibrils take some time to be dissolved at 80°C, even if there is a large excess of solvent. As aforementioned, all the microfibrils of a given *Valonia* cell wall fragment do not interact with the solvent at the same rate, since in a given preparation, there is at the same time a distribution of swollen states, as illustrated in Figures 1b-1c. Such distribution may be related to the perfection of the initial microfibrils where the presence or the absence of crystalline defects may govern or not the extent of swelling at a given time. In related systems where *Valonia* cellulose was swollen in strong aqueous NaOH solutions, it was shown that no swelling could occur unless some defects could first be created by some acid hydrolysis (23). It was thus clear that in order to penetrate into the cellulose lattice, the aqueous NaOH had to start from some crystalline defect. A similar mechanism

must take place here, but at a much faster rate as NMMO. H₂O is much more aggressive than NaOH to break the hydrogen bonds of cellulose.

Another somewhat unexpected result of this study is that in their interaction with NMMO. H₂O, *Valonia* microfibrils do not break down into shorter segments, but undergo only a longitudinal splintering. This observation is an indication that the cellulose chains remain intact during the whole process and that the swelling forces are not strong enough to break the cellulose molecules. In fact, cellulose is subject to severe oxidation and chain scission when interacted with NMMO alone, but the use of the antioxidant of *n*-propyl gallate protects the molecules and prevents any cellulose chain scission (24). The use 2% of this antioxidant with respect to NMMO. H₂O, may therefore explain why no cellulose chain and consequently no microfibril was split into short elements during the swelling process.

The spectacular images of *Valonia* microfibrils swollen in NMMO. H₂O can be compared with those of other systems where the microfibrils were also swollen under the action of specific intra-crystalline swelling agents. Images somewhat reminiscent of the one presented here were obtained when *Valonia* cellulose was converted into cellulose III_I by addition of ethylenediamine. In such a case, as in the present one, each microfibril became swollen without intermingling with its neighbors and one observed a longitudinal splintering of the microfibrils (16), but much less pronounced than the one shown in the present case. In fact, the complex of cellulose with ethylenediamine is highly crystalline and possesses a fixed stoichiometry based on one ethylenediamine molecule per glucosyl residue (25). This incorporation of ethylenediamine into the cellulose lattice leads to its lateral expansion of about 1.5 time, and thus to an equivalent increase of the diameter of the microfibril (26). This increase, which is about half of what is observed in Figure 1d, explains why the

ethylenediamine-swollen microfibrils do not show the unique open sub-fibrillated structure, which is shown here.

In the case of swelling with concentrated aqueous NaOH, only acid treated *Valonia* microfibrils could be modified (23, 27). After such a treatment, the swelling of individual microfibrils in a pattern similar to that of ethylenediamine or NMMO. H₂O was not observed. In the case of compact cell wall fragments, the action of NaOH leads to an extensive intermingling of the microfibrils, but no sub-fibrillation could be observed (27). In the case of individual microfibrils, the swelling action corresponded more to a stripping of the cellulose chains located at the surface of the microfibrils while the core remained intact (23). Again, the swelling pattern in concentrated NaOH was substantially different from the one observed here, which, so far, appears to be unique in the field of cellulose.

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Caption for the Figures

Figure 1. Negatively stained images of: **1a**: initial *Valonia* microfibrils; **1b-1d**: aspects of *Valonia* microfibrils after swelling for 10 minutes in NMMO. H₂O at 80°C. Figures 1a-1d are at the same magnification.

Figure 2. Electron diffraction diagrams of: **2a** fragment of an initial bundle of *Valonia* microfibrils with vertical axis; **2b**: as in 2a, but treated for 10 minutes in NMMO. H₂O at 80°C (sample morphology as in Figure 1b or 1c. **2c**: as in Figure 2b, but from a sample bundle of such as in Figure 1d. **2d**: sample of *Valonia* microfibrils treated for 4 hours in NMMO. H₂O at 80°C. The sample was totally dissolved and reprecipitated.

Figure 3. Schematic representation of the swelling of one *Valonia* microfibril subjected to NMMO. H₂O at 80°C for a few minutes. This sketch corresponds to the images shown either in Figure 1b or 1c.

Figure 1

Figure 2

Figure 3