Lattice imaging of ramie cellulose

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Electron microscopical lattice images were obtained with microcrystalline cellulose from ramie. Two lattice spacings of 0.40 and 0.60 nm, both parallel to the fibre axis, were observed. The width of the crystalline region, as determined from lattice images ranged 3–11 nm, corresponding well to that of rod-shaped particles observed by negative staining. The discrepancy between these values and the size of elementary microfibrillar unit in higher plant cellulose was interpreted by the possible coagulation of crystallites caused by acid treatment to form a continuous crystalline order.

(Keywords: electron microscopy; lattice image; cellulose)

Introduction
The lattice imaging by high-resolution electron microscopy is becoming an important tool in studying the ultrastructure of cellulose and related crystalline biopolymers1–6. The most crucial factor for the success of this technique is the size of crystallite to be imaged, by which the contrast of the lattice image is strongly affected. Apparently, this is the reason that lattice images have been obtained mostly with highly crystalline algal celluloses.

We have shown that lattice imaging is possible with bacterial cellulose which has considerably smaller crystallite size than algal celluloses6. This material, however, is still much more crystalline than higher plant celluloses. We here report the first success of lattice imaging of cellulose from a vascular plant.

Experimental
Cellulose sample. A purified ramie cellulose was disintegrated into microcrystalline particles by treating it with boiling 20% HCl for 10 min. The material was thoroughly washed with water through repeated centrifugation. The final suspension was ultrasonicated for a better dispersion, and the fine particles that remain suspended after sedimentation of large particles were used.

Electron microscopy. The procedure was basically the same as reported earlier (see ref. 6 for details).

(a) Specimen grid. The sample was mounted on an ultrathin carbon film supported by platinum–carbon reinforced Formvar microt.

![Figure 1](https://example.com/figure1.png)

Microcrystalline ramie cellulose as negatively stained with uranyl acetate. Note the variation in width of particles: a = 4, b = 6 and c = 11 nm

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Figure 2  A 0.40 nm spacing lattice image of ramie cellulose and its computer processing. (a) Original picture, (b) two-dimensional Fast Fourier Transform (FFT) of (a), processed by a high-pass filter to enhance the 'diffraction' spots (marked with arrows). The large cross-like pattern is originated from the straight edges of the windowed area. (c) Median high-pass filtered image of (b). (d) Vertical low-pass filtering applied to (c). Random background is evened out. The arrows mark eight well-defined fringes, corresponding to a width of 2.8 nm. (c) and (d) show the identical field on the CRT screen.

(b) Electron microscope. Philips EM420 equipped with a low dose unit.

(c) Image intensifier. GATAN TV imaging system (GATAN Corp., USA) was introduced for precise focusing and stigmation which were carried out by observing the granularity of the carbon film.

(d) Recording film. Kodak TMAX 400 film was used with a 35 mm camera. This film was recently released by Eastman Kodak as the successor to Tri-X Pan which was used in our previous study.

(e) Development. Microdol-X (full strength), 10 min at 24°C.

(f) Electron–optical conditions. Electron source: tungsten hairpin filament; acceleration voltage: 120 kV; condenser aperture: 15 or 30 μm; spot size: positions 3–5; objective aperture: 50 μm; magnification: ×15000–25000; electron beam density for recording: 10–20 electrons nm⁻² s⁻¹; exposure: 10 or 20 s.

(g) Digital image processing. Zeiss-Kontron IBAS system was used for the enhancement of lattice images. This system provides various kinds of operations, including filters and a two-dimensional Fourier transformation, applicable as a digitized image stored in 512 × 512 pixels.

Results and Discussion

Many electron-microscopical studies have shown that higher-plant cellulososes contain a fundamental fibrillar unit of 2–5 nm wide⁷⁻¹²*. The hot acid treatment is known to preferentially hydrolyse the amorphous or less-ordered regions of cellulose, thus leaving minute crystallites as a colloidal suspension. These crystallite particles are expected to have a dimension corresponding to the structural unit in native microfibrils. However, Figure 1, an electron micrograph

* Though this unit is often denoted as 'elementary fibril' after Frey-Wyssling, this notation should not be perpetuated, at least in its original sense, because it was proposed as the universal structural unit in all types of native cellulose. The lattice imaging of cellulososes from Vigna¹⁻³, Boerhavesia and Acetobacter⁶ has shown that the cross-sectional size and shape of crystalline unit are widely variable with the cellulose producing organism.
of negatively stained microcrystalline ramie cellulose, reveals an unexpected feature with the lateral size of crystallites. The width of rod-shaped particles range c. 4 (arrow a) to 12 nm (arrow c), thus considerably exceeding the width of the supposed microfibrillar unit. Lattice images of the same sample should give some information about the crystalline nature of these particles.

Figures 2 and 3 show the corresponding lattice images together with the results of image processing. Figures 2a and 3a are original images directly printed from the photomicroscopic negatives which were obtained from the electron micrographs with a linear magnification of c. 20. Because of the small crystallite size, the lattice fringes are barely visible on the original pictures. One can recognize them by viewing at the picture at a low angle and parallel to the lattice line. (All pictures are arranged to make the lattice lines vertical.) The two-dimensional Fourier transformation by computer clearly showed the corresponding ‘diffraction’ patterns (Figure 2b), thus confirming the presence of lattice fringe. After thus locating the lattice fringes, various combinations of enhancing operations were applied to them. (See, e.g. ref. 13, for details about the digital image processing.)

Figures 2c, d and 3b are results of such operations. The fields reproduced from the CRT screen (c. 440 x 440 pixels) closely correspond to those of the original prints. As a preliminary noise reduction, a median high-pass filter, which is the subtraction of median low-pass filtered image from the original, was applied. Figure 2c shows the result of this operation as applied to Figure 2a. The vertical low-pass filtering, which is to average the grey value of pixels in vertical direction, even out the random granular noise, and only vertical lattice structures are extracted as seen in Figures 2d and 3b. With these pictures of enhanced lattice fringes, one can determine the width of the crystallite. The widths determined from these figures are: 3 nm for the 0.4 nm lattice in Figure 2d, and 11 nm for the 0.60 nm lattice in Figure 3b.

Because the observed lattice spacings of 0.40 and 0.60 nm agree to those corresponding to equatorial reflections of cellulose I, these lattices obviously lie parallel to the long (fibre) axis of the rod-shaped particles shown in Figure 1. The occurrence of 11 nm wide lattice shows that at least some of the thick particles such as seen in Figure 1 are essentially single crystals. Because the basic microfibrillar unit of higher plant cellulose is believed to have the width of 2-5 nm, the occurrence of crystallites as thick as 11 nm requires an explanation.

One possibility is that the microfibrillar unit is not very uniform in width, showing some variation between or within each microfibril; however, this is not likely according to previous studies with thin sectioned or disintegrated materials, which showed fairly uniform microfibril width.9,12 Another possibility is that the crystallite size actually increases by hot acid treatment in the preparation of microcrystalline cellulose. This seems possible in the light of the so called ‘recrystallization’ of cellulose on acid hydrolysis.14,15 A corroborating observation has been reported in the study of explosion pulping process.16 Further studies, including the lattice imaging of non-acid-treated samples, are necessary for the understanding of the crystalline morphology in native microfibrils.

As far as the resolution and extent of the present lattice images go, there is no periodic disruption of lattice structure across the fibre axis. This observation is consistent only with the extended chain structure in the crystal, and not with a model involving periodic chain foldings.17

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References

Ruthenium tetraoxide staining technique for transmission electron microscopy of segmented block copolyetherester

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Lamellar morphology of melt-crystallized polyetherester (PBT-PTMO) has been investigated by extending the technique of ruthenium tetraoxide ($\text{Ru}_4\text{O}_8$) as a staining agent for the observation of the cast films using transmission electron microscope (TEM). The excellent image contrast obtained indicates that the $\text{Ru}_4\text{O}_8$ staining technique is very effective and simple for the study of the lamellar structure of segmented block copolyetherester.

(Keywords: lamellar morphology; RuO$_4$ staining; transmission electron microscopy; segmented block copolyetherester)

Introduction

The mechanical properties of the polymers depend on their supermolecular structure, formed as the result of processing conditions. From this view point, morphological knowledge of the lamellar structure is indispensable for a fundamental understanding of the properties of segmented block copolyetherester. The transmission electron microscope (TEM) is an established instrument for the characterization of the lamellar structure of crystalline polymers at a high level of resolution. However, it is often necessary to enhance image contrast for polymers by using a staining technique. A suitable image contrast enhancing stain for segmented block copolyetherester has been lacking. Cella$^1$ was successful in revealing a domain structure by using the phosphotungstic acid as a staining agent for segmented copolyetherester, but it did not generate sufficient resolution of the domain boundaries and other details. Wegner et al.$^2$ observed the detailed lamellar structure of segmented block copolymers by a two-step staining method with OsO$_4$/allyamine, but this method is more difficult to use.

Trent et al.$^3$ recently showed that ruthenium tetraoxide ($\text{Ru}_4\text{O}_8$) is an effective staining agent for TEM study of the morphology in both saturated and unsaturated polymers systems. Montezinos et al.$^4$ and Sano et al.$^5$ reported that polyethylene and polypropylene could be stained by $\text{Ru}_4\text{O}_8$ and Cao et al.$^6$ succeeded in resolving the lamellar structure of poly(tetramethylene oxide glycol 2,6-naphthalene dicarboxylate) by the $\text{Ru}_4\text{O}_8$ vapour staining technique, but a study of $\text{Ru}_4\text{O}_8$ as a staining agent for TEM observation of segmented copolyetherester has not been found yet in available literature.

In this paper the applicability of the $\text{Ru}_4\text{O}_8$ staining technique to the lamellar morphology of segmented block copolyetherester will be investigated.

Experimental

Sample preparation. A sample of copolyetherester consisting of poly(tetramethylene terephthalate) (PBT) as a hard segment and poly(tetramethylene oxide) (PTMO) as a soft segment, with total weight fraction of 0.72 of the hard segment and an average degree of polymerization of 14 for the soft segment, was used in our investigation. The melting point of the sample determined by d.s.c. measurement is 216°C.

A dilute solution (0.5%) of the PBT-PTMO in 1,1,2,2-tetrachloroethane was carefully dropped and uniformly spread onto a carbon film (thickness about 200 Å) supported by a mica slice at room temperature. The sample was treated at 220°C for 10 min after the solvent was evaporated, then annealed at 184°C in a nitrogen atmosphere for 6 h.

Figure 1 Electron micrograph of a spherulite of PBT-PTMO film melt-crystallized at 184°C for 6 h.