# Directionality of the fibre *c*-axis of cellulose crystallites in microfibrils of *Valonia ventricosa*

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The directionality of the *c*-axis (fibre axis) of the cellulose crystallites in the cell wall of *Valonia ventricosa* has been elucidated using tilt experiments, electron diffraction in selected areas and micro-diffraction on ultrathin sections. It is shown that each lamella of the cell wall contains the two directions 'up and down' for the *c*-axis, while for one single microfibril, this *c*-axis is unidirectional, as for a single crystal. A direct consequence of this last result is that the 3.5 nm elementary microfibrils, which are generally thought to be the basic morphological units of plant cell walls, can be considered to occur as separate entities within a microfibril of *Valonia ventricosa* only if they have the same sense and the defects at the boundaries are small.

**Keywords** Cellulose structure; crystallites; fibrils; *Valonia ventricosa*; polarity; electron diffraction

## INTRODUCTION

The cell wall of *Valonia ventricosa* is known for its multilamellar structure[1]. Each lamella contains several layers of parallel microfibrils. A uniplanar orientation for the cellulose crystal in different Valonia species has been found by X-ray diffraction[1,2] with the $(2\bar{2}0)$* planes preferentially parallel to the cell wall surface. These authors also found that the molecular axis, *c*, has two major orientations at an angle less than 90°, coinciding with the two orthogonal arrays of micrfibrils. In addition they noted that, in each parallel array of microfibrils, the *c*-axis presents two directions at 180° to each other. Thus, if one *c* direction is 'up', the other is 'down'.

The purpose of this paper is to establish whether such opposite directions for the *c*-axis occur within one lamella of the cell wall of *Valonia ventricosa* and within a single microfibril. The techniques of selected-area electron diffraction and micro-diffraction on ultrathin sections of Valonia were used.

## EXPERIMENTAL

### *Materials*

Samples of *Valonia ventricosa* preserved in aqueous formaldehyde were purified successively in aqueous sodium hydroxide, distilled water, hydrochloric acid and distilled water as described by Gardner and Blackwell[4].

### *Specimen preparation*

Fragments of Valonia cell were delaminated carefully in water with needles and the resulting lamellae or layers of microfibrils were mounted on carbon coated microscope grids. When sections of the Valonia cell wall were needed, with one of the two major orientations of the microfibrils transverse to the sections, the procedure described previously[5] was used.

### *Electron microscopy*

A transmission electron microscope (Philips EM 400T) was used. The accelerating voltage was 120 kV and to maintain the crystallinity of the specimen during observation, the microscope was operated in low intensity beam conditions by overfocusing the first condenser lens.

Two electron diffraction techniques were used, selected-area diffraction and micro-diffraction. In selected-area diffraction, the area of interest was selected by a diffraction aperture and was 0.5 $\mu$m in diameter or larger. In micro-diffraction, only the area of interest was irradiated by the electron beam and the size of this area could be as small as 40 nm in diameter. The second condenser apertures were small (5 $\mu$m and 2 $\mu$m) to reduce the beam damage and beam convergence. When required, condenser astigmatism was introduced to record micro-diffraction from a suitably elongated area.

For tilt experiments a special rotating holder was used which, in conjunction with the high-tilt goniometer, allowed tilt in the range of $-60°$ to $+60°$ from any chosen axis in the horizontal plane.

## RESULTS

By delaminating the cell wall, it is easy to isolate a part of one lamella composed of several layers of parallel microfibrils. Such a preparation is shown in *Figure 1a*. The electron diffraction pattern of the selected area indicated by the circle is shown in *Figure 1b*. This pattern oriented with respect to the direction of the microfibrils in the photomicrograph is the fibre pattern of cellulose I. The meridian is vertical and the equator horizontal. From the centre, the three main equatorial reflections can be indexed as $(2\bar{2}0)$, (220) and (040) respectively. These reflections correspond to crystallites in Bragg orientation with respect to the electron beam. From their relative intensities it is apparent that only a few crystallites have the $(2\bar{2}0)$ plane in Bragg orientation while many have the (220) and (040) planes in such a position. For diffraction by electrons the Bragg angle for these equatorial planes is

* Throughout this paper, the indexing of native cellulose refers to the 8 chain unit cell as proposed by Gardner and Blackwell[3], with *c* as the fibre axis

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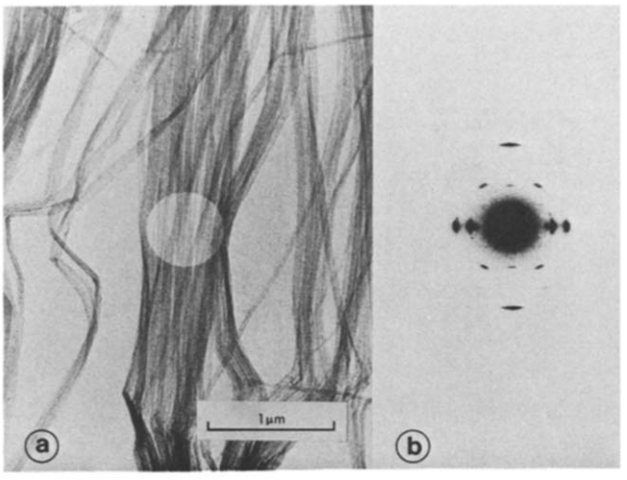


*Figure 1* (a) Bright field electron micrograph of cellulose microfibrils of *Valonia ventricosa* prepared by delamination of the cell wall. (b) Electron diffraction pattern from the area shown in *Figure 1a*, properly oriented with respect to the image

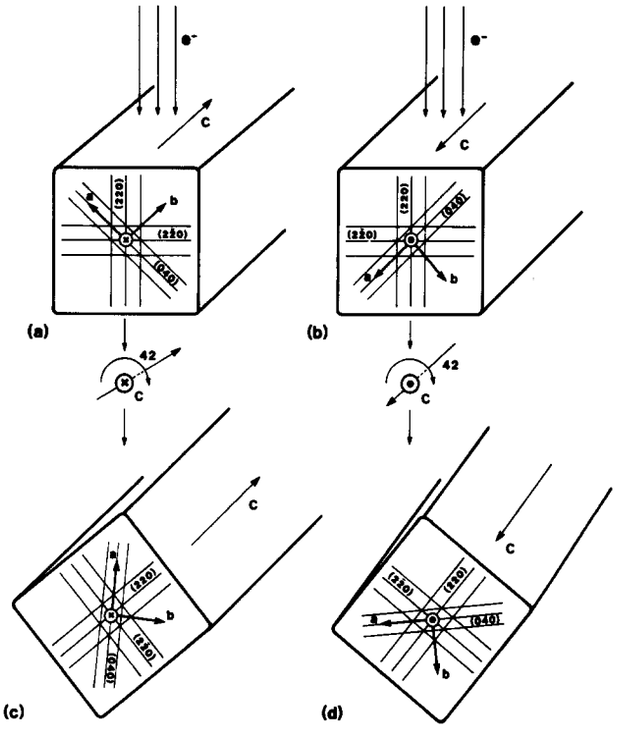


*Figure 2* (a) and (b) Schematic diagram of a cellulose microfibril seen in cross-section, with the two different dispositions of the equatorial planes according to the directionality of the *c*-axis. (a) *c*-axis is down; (b) *c*-axis is up; (c) and (d) the same as (a) and (b) but after rotation of 42° about the *c*-axis

almost 0°, i.e., they are parallel to the electron beam. Accordingly it follows from elementary crystallographic considerations that in this multi-layer preparation, most of the (2$\bar{2}$0) planes are parallel to the surface of the supporting carbon film, while the (220) planes are essentially perpendicular to the carbon film and parallel to the electron beam. These electron diffraction results thus support the previous findings by X-ray diffraction[1,2].

The directionality of the *c*-axis of the microfibrils was then elucidated by tilt experiments. *Figures 2a* and *2b* shows two cellulose crystallites in cross-section, with their respective crystallographic axes corresponding to the preferential orientation mentioned previously. In *Figure 2a*, the *c*-axis is pointing 'down' and in *Figure 2b* it is pointing 'up'. The (2$\bar{2}$0) and (220) planes have the same orientation in the two Figures while the orientation of the (040) plane changes by 90°. If only the 'up' direction for the *c*-axis occurs (*Figure 2b*), it should be possible to remove the (040) plane from the Bragg position by tilting the sample around the *c*-axis in a direction which positions this plane perpendicular to the electron beam (see *Figure 2d*). In such an alignment it would be impossible to have the (040) planes in Bragg orientation (parallel to the electron beam) even with a high degree of misorientation. If, however, the two *c*-directions occur, it would be difficult to exclude completely the Bragg position for the (040) plane because, when one series of the planes corresponding to one direction for the *c*-axis is perpendicular to the electron beam, the other must be parallel to it, i.e., in the Bragg position (see *Figure 2c*).

A series of electron diffraction diagrams obtained by sequential tilting around the *c*-axis is shown in *Figure 3*. In terms of intensity, at 0° tilt angle, the (2$\bar{2}$0) reflection is almost totally absent, while the (220) reflection is strong. Inversely, at + or −60° tilt, the (2$\bar{2}$0) reflection is strong while the (220) one is very weak. In contrast, the (040) reflection is always present, but with a stronger intensity at + or −45° tilt. These results thus confirm that the *c*-axis has opposite directions at the level of organization of a single lamella of the cell wall.

The 'up and down' directionality of the *c*-axis in a single lamella was then confirmed by micro-diffraction on ultrathin sections of the *Valonia ventricosa* cell wall. The section in *Figure 4* shows the presence of successive lamellae. The cellulose microfibrils inside each lamella are parallel and some of them are exactly perpendicular to the plane of observation and thus parallel to the electron beam. This photomicrograph has been interpreted in a previous paper[5] and the black spots represent the cellulose crystallites seen in transverse section. The group of microfibrils in the centre of the Figure represents the area in which the micro-diffraction was recorded. A pronounced astigmatism in the first condenser lens was used, as described previously. The corresponding micro-diffraction is shown in *Figure 5a*, oriented with respect to the image. The diagram shows clearly groups of reflection spots indexed as shown in *Figure 5b*. The results again confirm the preferential orientation previously found[1,2] with the (2$\bar{2}$0) planes parallel to the surface of the cell wall. The presence of strong (220) reflections means that some cellulose crystallites in this area are slightly tilted in the plane of the lamella. Indeed, in such a condition the (2$\bar{2}$0) planes remain in Bragg orientation (parallel to the electron beam) while the other equatorial planes are out of the Bragg orientation. The presence of the two positions for the (040) planes indicates clearly the presence of the two 'up and down' directions for the *c*-axis within one lamella.

To record a diffraction pattern for one single microfibril in cross-section, the spot size of the beam was reduced to 40 nm. This was found to be the smallest spot size which allowed sufficient time for recording a diffraction pattern before damage. Several patterns were successfully recorded and one of them is presented in *Figure 6a*, oriented with respect to the image of *Figure 4*. The pattern consists of a lattice of sharp reflection spots similar to those that would be obtained with perfect single crystals. The

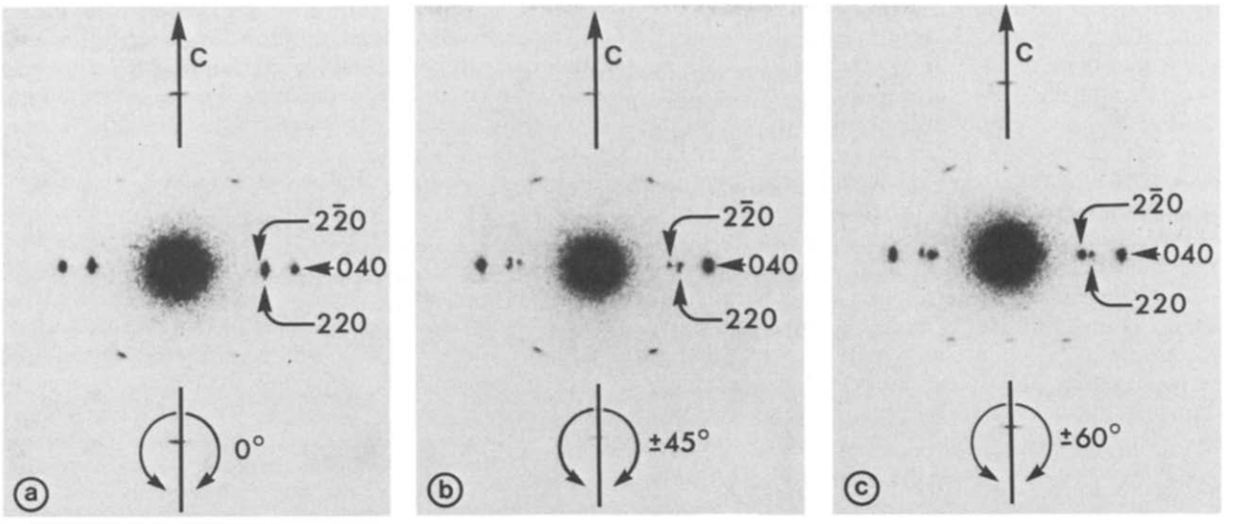


*Figure 3* Series of electron diffraction diagrams obtained by sequential tilting around the *c*-axis (microfibril axis) from an area similar to that shown in *Figure 1a*

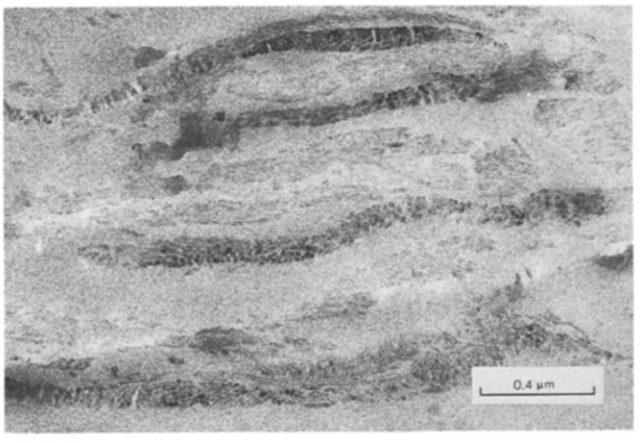


*Figure 4* Bright field electron micrograph in the Bragg contrast mode of a transverse ultrathin section of *Valonia ventricosa* cell wall. The cell wall surface is parallel to the horizontal of the picture

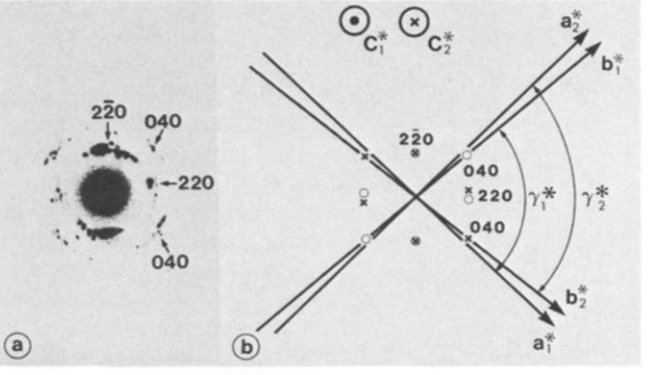


*Figure 5* (a) Micro-diffraction of the group of microfibrils in the center of the print in *Figure 4* properly oriented with respect to the image. (b) Schematic of the pattern showing the indexation of the groups of reflection spots. *O*, reflections corresponding to the $a_1^*$, $b_1^*$, $c_1^*$ reciprocal unit cell; and *X*, reflections corresponding to the $a_2^*$, $b_2^*$, $c_2^*$ reciprocal unit cell

indexing of this pattern is shown in *Figure 6b*. It represents the reciprocal lattice of the equatorial (*hkO*) plane of a cellulose I single crystal, which indicates one unique direction for the *c*-axis in the cellulose crystallite.

## DISCUSSION

The results confirm for *Valonia ventricosa* the preferential orientation of the microfibrils shown by Preston[1] and by Tanaka and Okamura[2] with the (2$\bar{2}$0) planes parallel to the surface of the cell wall.

Also confirmed in the present work are the two 'up and down' directions for the *c*-axis. This bidirectionality has been demonstrated previously for the whole cell wall by Preston[1] and by Tanaka and Okamura[2]. In the present work, this effect was found in each lamella of the cell wall. The important new result, however, is the finding that, for one single microfibril, the *c*-axis is unidirectional. The result has relevance to the controversy concerning the presence or not of 3.5 nm elementary microfibrils within one microfibril of Valonia as was proposed by Frey-Wyssling *et al.*[6]. It has been shown by dark field electron microscopy[7-9], by negative staining on cross-sections[10]

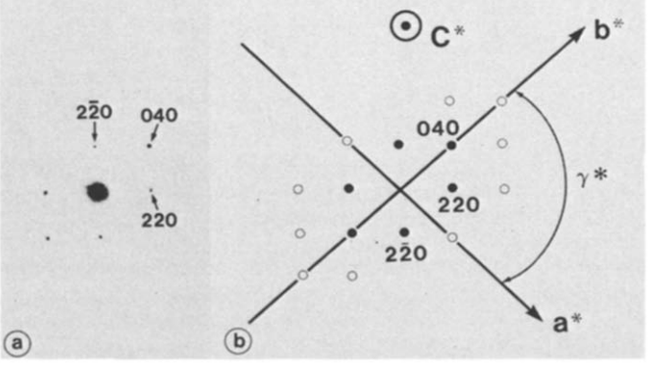


*Figure 6* (a) Micro-diffraction pattern of one single cellulose microfibril in cross-section, similar to those presented in *Figure 4* and oriented with respect to the image. The spot size was reduced to 40 nm. (b) Schematic diagram representing the indexing of the pattern. The open circles represent reflections visible on the original negative

and by Bragg contrast in bright field on cross-sections[5], that the cellulose microfibril in Valonia has the appearance of a single crystal. However, it would be possible for the 3.5 nm elementary microfibrils within the microfib-

ril to be packed in such perfect registry that they behave together as a single crystal. The results of the present work go further and demonstrate clearly the presence of one unique *c*-axis, which is a necessary condition to be a single crystal. It seems, therefore, that if the 3.5 nm elementary microfibrils occur as separate entities in a microfibril of *Valonia ventricosa*, they have the same sense, and the defects of the boundaries must be small[11].

This polarity of the cellulose crystallites has, however, no direct relevance to the polarity of the chains within the unit cell of the cellulose I structure. The parallel or antiparallel arrangement of the molecular chain still needs to be established. Nevertheless, it may be expected that the enzyme system producing the cellulose microfibril would always make the microfibril with the same *c*-axis direction. This suggests, therefore, that enzymes move in opposite directions in spinning the various layers of one lamella of Valonia and could be explained by the mechanism of wall formation in plant cells proposed by Chafe[12], in which a microfibril is generated by a moving granule (enzyme complex) which travels up and down the long axis of the cell. It could also be explained by the hypothesis proposed by Brown[13] for cell wall biogenesis of *Oocystis apiculata*, in which the microfibrils synthesis is associated with a paired enzymatic complex, each complex moving in opposite direction as soon as the initiation of microfibril synthesis starts. If the microfibril is actually an aggregate of elementary fibrils it may be expected that they would be synthesized by the concerted efforts of groups of enzyme complexes.

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