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Note

Interconversion of the Iα and Iβ crystalline forms of cellulose by bending

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Abstract

Bending cellulose in a plane normal to the hydrogen-bonded sheets of chains causes a longitudinal displacement of the sheets with respect to one another. The magnitude of this displacement is shown to be sufficient to interconvert the Iα and Iβ forms of cellulose within a bending angle of 39° when the curvature of the sheets of chains comprising the microfibril is modelled as a series of concentric circular arcs. Bending through an angle of 90° is more than sufficient to convert the Iα form into Iβ and back again. Cellulose microfibrils emerging from the cellulose synthase complex in the plasma membrane must bend sharply before they can lie parallel with the inner face of the cell wall. The scale of the changes induced by bending is sufficient to ensure that whatever crystal form would be expected from the geometry of the biosynthetic complex, it is likely be radically altered before the cellulose is incorporated into the cell wall.

Keywords: Cellulose; Crystallinity; Biosynthesis; Bending

1. Introduction

Natural cellulose is a mixture of two crystalline forms, Iα and Iβ, together with surface chains and less crystalline material [1,2]. The Iα and Iβ structures are now to a considerable extent understood from crystallographic experiments and modelling on the large, highly ordered cellulose microfibrils in the cell walls of algae such as *Valonia* [3–9]. The cellulose chain conformation is similar in both forms and the principal difference between them is the manner in which the chains are staggered longitudinally: this gives triclinic crystallographic symmetry to the Iα form and monoclinic symmetry to cellulose Iβ [4]. Both staggered arrangements can be present within the cross section of a single, crystalline microfibril, i.e., mixed crystals are possible [10].

In higher plants the cell walls contain thinner cellulose microfibrils than those of *Valonia*. Microfibrils in higher plants have a higher proportion of surface chains and are ordered over too short a range to permit effective crystallography [11]. Their ^{13}C NMR spectra show, however, that the Iα and Iβ forms are both present [2,12,13]. The proportions of the two forms vary according to the source of the plant material [1,14,15]. In the textile fibres cotton and ramie, which contain the largest crystalline units and little non-cellulose material, the Iβ form predominates. In conifer tissues, primary cell walls contain more cellu-

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lose Iα than Iβ and the order is reversed in the secondary cell walls, which contain different non-cellulose polysaccharides [16].

The cellulose synthase complex of higher plants has been partly characterised and *Arabidopsis* mutants in which it is defective are now known [17]. The synthase complex of higher plants contains a number of catalytic subunits and other peptides arrayed in a rosette-shaped particle embedded in the plasma membrane, so that a group of parallel, nascent cellulose chains is extruded simultaneously from the rosette [18].

Two alternative hypotheses may be advanced for the simultaneous presence of the Iα and Iβ forms of cellulose. The first is that cellulose Iα is synthesised by a different type of complex from cellulose Iβ, and within each synthetic complex the catalytic subunits are so arranged that the chains emerge in the characteristically staggered formation appropriate to either the Iα or the Iβ form. So far no evidence has appeared to support this idea. The second hypothesis is that the two crystal forms of cellulose result from events that occur after the synthesis of the cellulose chains. Consistent with this alternative, the Iα:Iβ ratio in bacterial cellulose can be altered experimentally by the addition of non-cellulose polysaccharides such as xyloglucans that are capable of binding to cellulose [19–21]. This would imply that the balance between the two crystal forms is not finally determined until after the chains have passed out of the synthetic complex, and that it is then sensitive in some way to the immediate environment of the microfibril. However no molecular mechanism by which this might happen has so far been suggested.

In this paper it is shown that bending can interconvert the crystalline forms of cellulose I and that their ratio is very sensitive to the angle through which the microfibril is bent. The first part deals with mechanical bending of microfibrils that have already crystallised in the Iα or Iβ form. In the second part this analysis is qualitatively extended to microfibrils forming at the plant cell membrane, when they must bend sharply to take their place within the cell wall. A quantitative description of this second process is not possible because it probably takes place before the crystal geometry is established.

2. Bending of microfibrils that are already crystalline

In both crystal forms of cellulose I the glucan chains adopt a flat ribbon (2_1 helical) conformation and are arranged in sheets [4]. The chains within each sheet are held together by a highly ordered system of hydrogen bonding, while it is widely held that each sheet adheres to the next by van der Waals forces. Although some details of the Iα structure are still unclear [6,8], the crystallographic symmetry of the two forms is such that the principal operation needed to interconvert them is to slide one pair of sheets longitudinally over the pair below by a distance equal to 0.53 nm, the length of a glucose residue.

Molecular dynamics studies [6,9] have shown that this interconversion is indeed feasible. It can be induced experimentally by annealing cellulose at a high temperature, when the Iα form is converted to the thermodynamically more stable Iβ form [3]. The rationale for the transformation lies in the nature of the bonding between the cellulose chains. Van der Waals forces can hold two molecular sheets together without preventing one from sliding along the other, provided that the adhering faces are flat enough to prevent short contacts between atoms from ensuing. Hydrogen bonds between two chains within a sheet, on the other hand, have more precisely defined geometry and maintain the spatial relationship between these chains with corresponding precision. Thus although the bonding energy between sheets (i.e., the energy required to pull the sheets apart in tension) is comparable with the bonding energy within each sheet [5,6], there is relatively little resistance to shear between sheets.

When a bar is bent, shear stresses are generated within it as the inner face becomes shorter than the outer face. Bending the structure is easier if these stresses can be dissipated. For example, leaf springs in the suspension of a road vehicle are designed to dissipate shear by allowing each leaf to slide past the one

below when the whole spring bends. It seems logical, therefore, that a similar sliding displacement might occur between sheets if a cellulose microfibril were bent in a direction normal to the sheets, i.e., normal to the [020] crystal plane in the Gardner and Blackwell [22] notation. The magnitude of displacements of this type is derived below for crystalline cellulose I.

If it is assumed that two double sheets of chains follow concentric arcs of radius r and $(r+\delta r)$, respectively, subtending an angle θ, (Fig. 1), the shear strain δh between them is given by Eq. (1):

$$\delta h = \theta \times \delta r \quad (1)$$

Note that in this model the displacement is dependent only on θ, the angle through which the microfibril is curved, and on δr, the spacing between the pairs of sheets. It does not depend on r, the radius of curvature.

In the case of the Iα→Iβ transition, $\delta r = b \sin\gamma = 0.78$ nm (in the Gardner and Blackwell notation for Iβ [22]) since pairs of sheets are sliding together. A longitudinal displacement of $c/2 = 0.52$ nm is required to convert Iα to Iβ so the bending angle is equal to:

$$\theta = \frac{\delta h}{\delta r} = \frac{0.52}{0.79}\ \text{nm} = 0.67\ \text{radians} = 39° \quad (2)$$

If, for example, a microfibril is bent through 90° the longitudinal displacement between each pair of sheets is 1.21 nm, enough to convert cellulose Iα to Iβ, back to Iα and somewhat further.

Eqs. (1) and (2) are based on a model in which the bending geometry is accommodated entirely by shear between the sheets of chains, and the length of each chain remains the same. In an alternative model the chains might lengthen in the outer part of the microfibril and shorten in the inner part. Which of these models is more realistic? This can be assessed by calculating the energy input required in each case.

For illustration (Fig. 2) the calculation is based on a segment of a hypothetical microfibril consisting entirely of cellulose I, with 5 nm square cross section (approximately the size of a cotton microfibril), bending through 39° in the plane of its diagonal on a 25 nm radius. This gives a length of 17 nm. The energy input required to bend the individual chains is neglected, as it is essentially the same in each case.

Hardy and Sarko [6] showed that the activation energy required to slide the chains past one another in the cellulose Iα⇔Iβ transition was about 4 kJ mol^{-1} of 2-chain unit cells. Since the 17 nm segment contains 630 unit cells, the energy input required is 4 kJ $mol^{-1} \times 630 = 2500$ kJ mol^{-1}, or (dividing by Avogadro's number) 4×10^{-18} J per fibril segment.

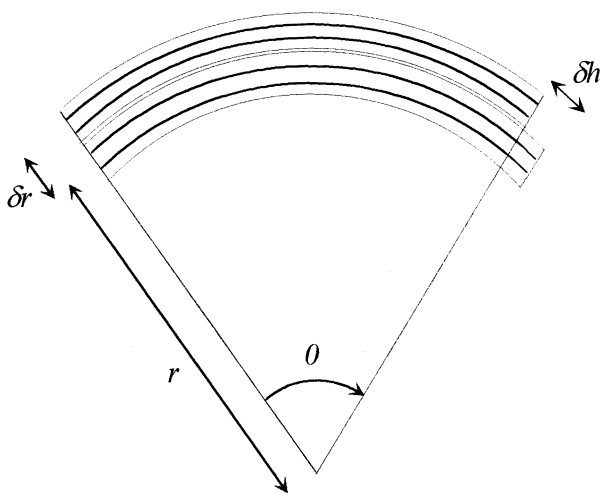

Fig. 1. Geometry of bending and the linear displacement between sheets of chains that is induced by it.

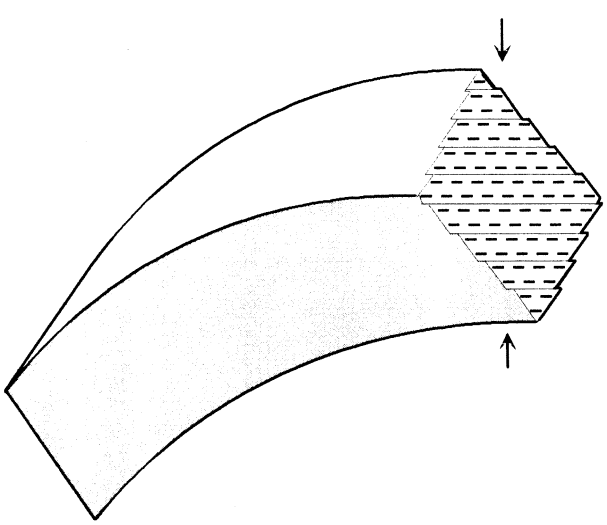

Fig. 2. The model microfibril segment used in the energy calculations; 17 nm long, 5 nm square, bent through 39° on a radius of 25 nm. This induces a relative linear displacement of the outer and inner chains, sufficient to interconvert the Iα and Iβ forms of cellulose within the length of the segment. The positions of the chain ends, if the microfibril segment were straight, are denoted by arrows.

This energy input required to bend the microfibril by stretching the outside and contracting the inside chains can be calculated directly from the tensile modulus E of cellulose I using Eq. (3) for a square beam under three-point bending stress in the plane of its diagonal:

$$\Delta = Fl^3/(4Eb^4) \quad (3)$$

where Δ is the deflection under applied force F and the span and side of the beam are l and b, respectively. Using the tensile modulus $E = 136 \times 10^9$ N m^{-2} given by Kroon-Batenburg and Kroon [8], the energy input $F\Delta$ is calculated as 1.5×10^{-16} J segment^{-1}.

The approximations involved in these calculations are considerable. For example, the activation energy for the cellulose Iα⇔Iβ transition is sensitive to the exact modelling parameters used, while surface chains may differ from cellulose I in their tensile modulus. However the calculated energy input for the sliding model is two orders of magnitude lower than for the other model, so the sliding model is more likely to be a realistic approximation, at least for microfibrils of the width considered here. Thin fibrils with a greater proportion of surface chains may behave in a more complex way.

The calculations above suggest that cellulose microfibrils are indeed capable of bending in a manner that would interconvert the Iα and Iβ forms of cellulose, and that the interconversion is completed within a bending angle of about 40°. Such relatively gentle bending may be seen in typical micrographs of cellulose microfibrils in plant cell walls.

3. Bending during microfibril formation

In higher plants (unlike bacteria), when a cellulose microfibril emerges from the synthetic complex in the plasma membrane it must bend sharply through about 90° so that it can lie flat against the inner face of the cell wall. Eqs. (1) and (2) are not likely to give a quantitative description of this situation, although the same qualitative principles apply. The geometry of the (1→4′)-β-glycosidic linkage in a single glucan chain, in the 2_1 helical conformation characteristic of cellulose I, cannot accommodate tight bending in a plane normal to that of the glucose rings. Either the chain conformation or the plane of bending must be different. It follows that the close, regular chain packing of cellulose I cannot be established until bending is completed. Eq. (1) will then underestimate the slippage between chains since their spacing δr will be increased, to an uncertain extent which might reasonably be influenced by the presence of non-cellulose polysaccharides [19–21].

Without knowing how the catalytic subunits are staggered within the cellulose synthase complex, we cannot yet use the concepts presented here to relate the Iα:Iβ ratio in muro to the geometry of cellulose at the point of synthesis. What is more important is the magnitude of the effect of bending on the crystal structure. This effect is so large that whatever the staggered configuration in which a group of cellulose chains is synthesised, it will change radically when these chains bend and aggregate to make up a microfibril within the plant cell wall. The eventual configuration is sensitive enough to the intersheet spacing, prior to coalescence of the microfibril, to provide a potential explanation for variations in cellulose Iα:Iβ ratio modulated by the molecular environment within which the nascent microfibril bends.

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