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Variations in the fibre repeat between samples of cellulose I from different sources

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Abstract—A powder X-ray diffractometer was used to measure the fibre repeat in cellulose I with sufficient precision to detect variations between samples from different sources. The variations were correlated with the lateral dimensions of the crystallites and were attributed to different minimum-energy fibre repeats for chains in the interiors and on the surfaces of crystallites. Results were interpreted in terms of a model for internal mechanical stress in which the interior chains were under compression and the surface chains under tension to ensure identical fibre repeats for all chains. The model was used to extrapolate the fibre repeat to a value of 1.043 nm for a hypothetical, infinitely large crystal, and to 1.029 nm for a crystallite so narrow that all chains were exposed on surfaces.

Keywords: Cellulose I; Fibre repeat; X-ray diffraction

1. Introduction

Fibre repeat is defined as the length of the repeating unit in a chain of a fibrous polymer.[1] The repeating unit in cellulose I is a pair of β-(1→4)-linked D-glucosyl residues (Fig. 1).[2] Nine measurements of the cellulose I fibre repeat between 1967 and 1991 gave a mean value of 1.036 nm.[2] The standard deviation of 0.002 nm was larger than the experimental uncertainty commonly attached to diffraction experiments in the latter half of the 20th century. We set out to test whether there might be natural variations in the fibre repeat between samples from different sources. In particular, we sought variations that might be associated with disruption of hydrogen bonding at the surfaces of crystallites.

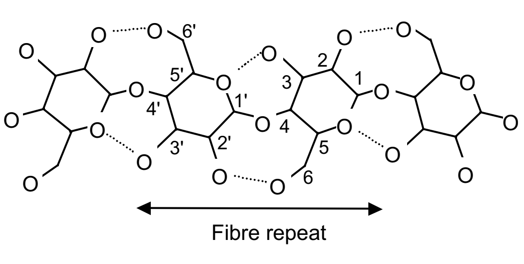

Figure 1. Representation of a chain segment from cellulose I, showing intra-chain hydrogen bonds as dotted lines.

Ioelovich and Larina studied 11 samples of cellulose I and found that the unit cell dimension a varied between 0.785 and 0.793 nm, while b varied between 0.816 and 0.821 nm.[3] These variations were much larger than experimental uncertainties, and were correlated with the lateral dimensions L of the cellulose crystallites. The unit cell dimension c, that is, the fibre repeat, did not show any detectable variations. The mean value was 1.034 nm, with a standard deviation of 0.001 nm. All 11 samples were prepared from cotton, flax or ramie.

We drew from a wider diversity of sources in our search for variations. Our experimental approach was similar to that of Ioelovich and Larina[3] in that we used a powder diffractometer to obtain wide-angle X-ray scattering (WAXS) diffractograms. An internal standard was mixed with powders, dusted on fibres

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before construction of fibre bundles or sandwiched between thin slices in laminated composites. Consistent use of the same internal standard throughout our work eliminated one of the sources of uncertainty in compilations of literature values for the cellulose I fibre repeat.

2. Results

We used the cellulose I (004) reflection because it was well resolved, whereas the (002) and (006) reflections could not be fully resolved from neighbouring reflections.[4] We chose MgO as the internal standard because of a strong reflection close to the cellulose I (004) reflection. WAXS diffractograms showed minor variations in the position of the MgO reflection, attributed to differences in sample geometry, but more obvious variations in the position of the cellulose I (004) reflection (Fig. 2).

Drying a cellulosic fibre can transfer compressive forces from the shrinking matrix to the embedded cellulose crystallites.[5] External forces applied to fibres can be sufficient to displace the (004) reflection by measurable amounts.[6] We tested the possibility of shrinkage-related displacements by obtaining WAXS diffractograms for moistened wood contained in a humid atmosphere (Fig. 3). The (004) reflection showed a negligible displacement when the moisture content was lowered from fully saturated conditions to oven-dried conditions, and the corresponding fibre repeat did not deviate by more than ±0.001 nm relative from the value of 1.034 nm reported in Table 1 for an air-dried sample with a moisture content of 10%.

Values of the fibre repeat were calculated for all 10 samples (Table 1) and are listed in descending order of sample-induced broadening β of the (200) reflection, used as an indicator for lateral dimensions of crystallites.[3]

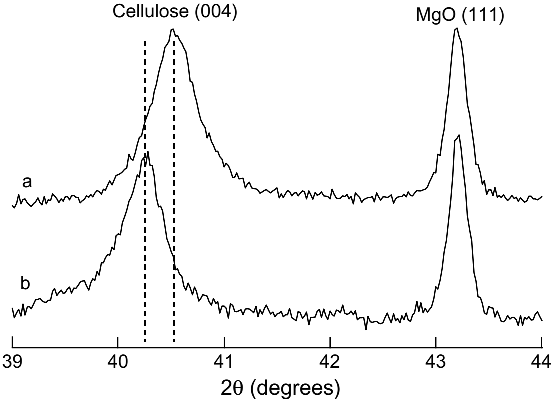

Figure 2. Portions of WAXS diffractograms of (a) sisal, (b) cotton. Vertical dotted lines mark the displacement between (004) reflections.

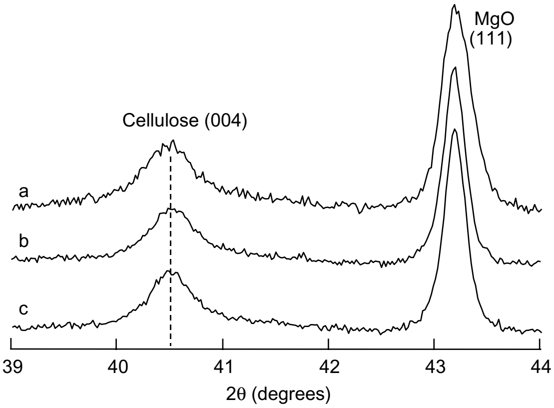

Figure 3. Portions of WAXS diffractograms of *P. radiata* wood with moisture contents of (a) 32%, (b) 17%, (c) <1%. A vertical dotted line marks the coincident (004) reflections.

Table 1. X-ray diffraction data for 10 samples of cellulose I

Sample	β (200) (degrees)	X[a]	Fibre repeat (nm)
P. radiata wood	3.31	0.362	1.034
Sisal twine	3.30	0.363	1.033
C. sativa wood	3.26	0.370	1.033
P. tenax leaf fibre	2.86	0.430	1.035
Jute twine	2.86	0.430	1.037
Linen thread	1.82	0.610	1.036
Commercial cellulose powder	1.33	0.706	1.037
Cotton thread	1.22	0.729	1.040
P. pachydermatina (tunicate)	1.19	0.734	1.039
C. coliformis (alga)	0.64	0.852	1.039

The uncertainty in the fibre repeat was typically ±0.001 nm.

[a] X = fraction of crystallite interior chains.

3. Discussion

3.1. Cellulose I_α and I_β

Variations in relative proportions of cellulose I_α and I_β allomorphs could not account for the variations in fibre repeat. We included in the study four samples previously characterised by solid-state ^{13}C NMR spectroscopy.[7] The two samples with the sharpest (200) reflections (Table 1) were an algal cellulose containing 57% I_α and 43% I_β and a tunicate cellulose containing 10% I_α and 90% I_β.[7] The fibre repeats were indistinguishable despite different proportions of allomorphs. Two of the broadest (200) reflections were observed for samples of wood. *Pinus radiata* contained 51% I_α and 49% I_β, *Castanea sativa* 25% I_α and 75% I_β.[7] Again, the fibre repeats were indistinguishable. These observations were consistent with those reported in an electron-diffraction study of algal cellulose, in that reflections from individual I_α and I_β domains indicated the same fibre repeat.[8] Recent studies,[9,10] based on X-ray and neutron diffraction, have indicated fibre repeats differing by slightly more than the experimental uncertainties, that is, 1.0400(6) nm for I_α-rich algal cellulose and 1.038(1) nm for I_β-rich tunicate cellulose. The difference of 0.002 nm remains significantly smaller than the difference of 0.007 nm between the shortest and longest fibre repeats in Table 1.

3.2. Model for internal stress

Recent experiments on celluloses II and III have indicated that both crystalline forms have a fibre repeat of 1.031 nm.[11,12] The experimental uncertainty was 0.005 nm in the former case, and not stated in the latter. This fibre repeat is significantly shorter than the fibre repeats for celluloses I_α and I_β, discussed above. It has been suggested that the hydrogen-bonding pattern characteristic of cellulose I does not extend to the surface.[13] Instead, the conformation of $-CH_2OH$ groups exposed on the surfaces might be closer to those found in celluloses II and III. We therefore propose a model for a cellulose I crystallite in which the theoretical minimum-energy fibre repeats for interior and surface chains, r_i and r_s are different. Specifically, we suggest that r_s is shorter than r_i and perhaps similar to values for cellulose II and III. Hydrogen bonding between surface and interior chains enforces a single value of r for all of the chains in a crystallite. This requirement for a single value of r places the surface chains in tension and the interior chains in compression.

Prediction of a value of r would require an explicit model for mixing domains of I_α and I_β along with any associated crystal defects. It would also require consideration of different force constants for contraction and expansion of interior and surface chains. We took a simpler approach based on the number of interior chains, expressed as a fraction (X) of the total number of chains in a cross-section through a crystallite. In the limit of a small crystallite, all chains are exposed on the surfaces, so $X = 0$ and $r = r_s$. In the limit of a hypothetical large crystal, $X \rightarrow 1$ and $r \rightarrow r_i$. For intermediate crystallite dimensions, r is an unspecified function of X.

We used the sample-induced broadening β of the (2 0 0) reflection to determine X, as follows. First we used the Scherrer equation to estimate the lateral dimensions L of crystallites[14]

$$L = K\lambda/(\beta\cos\theta), \tag{1}$$

where θ is the Bragg angle, λ is the wavelength of the radiation, and K is a constant that depends on the shape of the crystallite. A value of $K = 0.90$ is appropriate in Eq. 3 for the (1 1 0) reflection from cubic crystallites,[14] and is similarly appropriate for the (2 0 0) reflection from orthorhombic cellulose I if the crystallites are assumed to have nearly-square cross-sections with (1 1 0) and $(1\,\bar{1}\,0)$ planes exposed. Some authors introduce an additional term in the Scherrer equation to allow for line broadening due to defects, but the adjustment to the value of L is small and insensitive to crystallite size. Adjustments of 12–14% have been reported for the narrow crystallites in wood,[15] and 15–18% for the relatively large crystallites in algal cellulose.[16] We considered it inappropriate to assume one model for defects in order to test another model, so we chose to neglect the adjustment.

The surface chains occupy a layer approximately 0.57 nm thick,[17] so the proportion of crystallite interior chains is[17]

$$X = (L - 1.14)^2/L^2. \tag{2}$$

The values of X are tabulated in Table 1.

3.3. Best-fit fibre repeats

We started with the simplest function of X, that is, a linear expression

$$r = r_s + (r_i - r_s)X. \tag{3}$$

A least-squares fit of Eq. 3 to the data of Table 1, shown as a straight line in Figure 4, indicated $r_s = 1.029$ nm and $r_i = 1.043$ nm. The correlation coefficient was $R = 0.87$, indicating a confidence level of $P < 0.01$, and the root-mean-square deviation from the line was 0.0013 nm, that is, a value similar to the experimental uncertainty in each measurement of the fibre repeat.

We considered a model in which force constants differed for interior and surface chains, leading to a more complicated relationship between X and r. The force constant is greater for a cellulose I crystallite interior chain than for the chains in other crystalline forms.[18] It can be shown that allowing the force constant for the crystallite-surface chains to decrease, relative to crystallite interior chains, leads to curvature in the plot of r against X and extrapolation to smaller values of r_i and r_s at the two extremities. When curved plots were assessed, the root-mean-square deviation of points from the best-fit curve showed no significant improvement over the linear function.

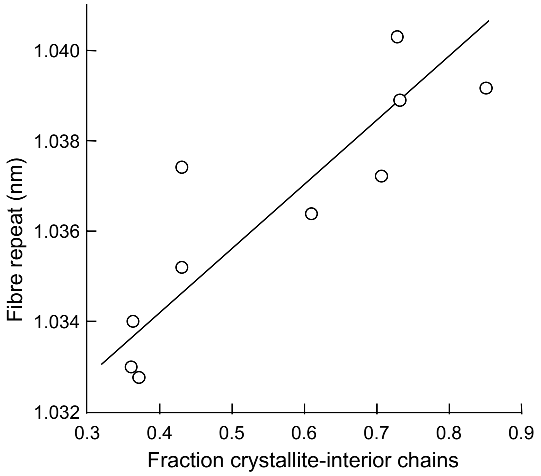

Figure 4. Fibre repeat distances measured for 10 samples of cellulose and the best-fit straight line through the data points.

The cell walls of green algae of the order Chlorophyta contain cellulose crystallites with cross-sectional dimensions larger than those in any other plant or animal. Typical lateral dimensions are in the range $L = 10$–25 nm.[19,20] Published measurements of the fibre repeat for celluloses from Chlorophyta show some scatter, but the most common values are between $r = 1.038$ and 1.040 nm.[3,9] These are indistinguishable from our value of $r = 1.039(1)$ nm for one of the Chlorophyta, *C. coliformis* (Table 1). The next-largest cellulose crystallites are found in animals of the order Tunicata. Typical lateral dimensions are in the range $L = 8$–10 nm.[19] Our value of r=1.039(1) nm for the tunicate *Pyura pachydermatina* (Table 1) is indistinguishable from a published value of $r = 1.038(1)$ nm for another tunicate species.[10] The crystallites in Chlorophyta and Tunicata have been generally assumed to be so large that surface effects might be ignored. Output from our model (Fig. 4) suggests that this assumption is not correct, and that a hypothetical macroscopic crystal of cellulose I would show a fibre repeat approximately 0.004 nm longer than published values. A curved plot of r against X might be more realistic, as discussed above, in which case the correction is better described as up to 0.004 nm.

The best-fit value of $r_s = 1.029$ nm is indistinguishable from the fibre repeat of 1.031(5) nm reported for cellulose II.[11] That similarity does not imply that cellulose I crystallite surfaces are similar to the interiors of cellulose II. We cannot reject the possibility that chains on surfaces of cellulose I crystallites differ from those in the interiors of any known crystalline form of cellulose.

4. Conclusions

We have shown that variations in the cellulose I fibre repeat did not arise from mechanical stress associated with drying samples, or from variations in the relative proportions of I_α and I_β allomorphs. The variations were consistent with a model in which the minimum-energy fibre repeat was 1.043 nm in crystallite interiors and 1.029 nm on surfaces. Hydrogen bonding between adjacent chains forces a uniform fibre repeat, so that interior chains are compressed and surface chains are stretched. We conclude that internal stress is present in the cellulose I crystallites of plants and animals.

5. Experimental

5.1. Samples for fibre repeat measurements

Samples of wood (*P. radiata* and *C. sativa*) were cut into fragments of dimensions <1 mm. Algal (*Chaetomorpha coliformis*) and tunicate (*P. pachydermatina*) tissue was air dried and ground in a Wiley mill to 40 mesh. Characterisation of cellulose I_α and I_β allomorphs in those four samples was described elsewhere.[17] Commercial cellulose powder (Sigma C6663) was used as received. These samples were mixed with MgO powder and spread on a sample holder made from a silicon wafer cut along (4 0 0) planes so that there were no background reflections in the region of interest. *Phormium tenax* leaf fibres were degummed with 4% NaOH at 100 °C for 2 h, washed and air dried. Cotton, linen, jute and sisal fibres were commercial products used as received. The fibres were dusted with MgO powder, gathered in bundles and sliced into discs approximately 0.6–0.8 mm thick. The fibres were retained in the bundles by bonding with poly(vinyl acetate) adhesive or by sealing them in 5-mm diameter heat-shrink plastic before slicing. The discs were placed on the silicon sample holder. This procedure orientated the fibres to improve the strength of the cellulose (0 0 4) reflection. The WAXS diffractogram of heat-shrink plastic showed no peaks in the region of interest.

5.2. Samples for linewidth measurements

Fibrous samples were cut into lengths of 1–2 mm; other samples were used as above. All were mixed with BN powder, used to determine the instrumental broadening, and pressed into discs.

5.3. Wide-angle X-ray diffraction (WAXS)

Diffractograms were obtained on two Philips PW1729 automated powder diffractometers with monochromated Co K_α radiation ($\lambda = 0.17903$ nm). A single instrument was used for all fibre repeat measurements reported in Table 1. Data acquisition was confined to the range $2\theta = 39$–$44°$. Despite lengthy data acquisition periods, the signal-to-noise ratio was seldom adequate to determine the positions of reflections to better than ±0.05°, corresponding to an uncertainty of ±0.001 nm in the fibre repeat. Entries in Table 1 were rounded accordingly. MgO powder was obtained by grinding an annealed single crystal. MgO has a cubic lattice with a mean edge dimension of 0.4211 nm and a standard error of 0.0001 nm calculated from data published by Broch,[21] Swanson and Tatge,[22] and three additional references cited in the latter work. The MgO (1 1 1) reflection was therefore expected at $2\theta = 43.21°$, and the scale was corrected for any deviations from this value. The standard deviation of the correction was 0.1°.

5.4. Reproducibility

Two samples of cotton were prepared, one bonded with poly(vinyl acetate) adhesive and the other contained in heat-shrink plastic. The two values of the fibre repeat were indistinguishable. The sample of *C. sativa* wood was run twice, and the results were indistinguishable.

5.5. Linewidth measurements

The uncorrected width B of the (200) reflection was measured following the method of Gjonnes and Norman.[23] The linewidth b of the adjacent BN peak was used to adjust results for instrumental broadening[24]

$$\beta^2 = B^2 - b^2. \tag{4}$$

5.6. Effect of water

These experiments were run on a second diffractometer. Three transverse slices of *P. radiata* wood were dusted with MgO powder and joined with small drops of methyl cyanoacrylate adhesive around the perimeter of each slice. The composite sample, approximately 1 mm thick, was laid on a silicon sample holder. A container was constructed from aluminum and sealed with a plastic film window to maintain humidity in the vicinity of the sample. A blank run showed no reflections in the region of interest. Water was added to the container to maintain 100% humidity when the fully saturated sample was run, and silica gel was added when the oven-dried sample was run. Moisture contents were determined by drying samples at 105 °C and reported as percent of total sample weight.

Acknowledgements

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References

1. Nyburg, S. C. *X-ray Analysis of Organic Structures*; Academic: New York, 1961; Vol. 1; p 341.
2. O'Sullivan, A. C. *Cellulose* **1997**, *4*, 173–207.
3. Ioelovich, M.; Larina, E. *Cellul. Chem. Technol.* **1999**, *33*, 3–12.
4. Nishimura, H.; Okano, T.; Asano, I. *Mokuzai Gakkaishi* **1982**, *28*, 659–668.
5. Barber, N. F. *Holzforschung* **1968**, *22*, 97–103.
6. Nishino, T.; Takano, K.; Nakamae, K. J. *Polym. Sci. Part B, Polym. Phys.* **1995**, *33*, 1647–1651.
7. Newman, R. H. *Holzforschung* **1999**, *53*, 335–340.
8. Sugiyama, J.; Vuong, R.; Chanzy, H. *Macromolecules* **1991**, *24*, 4168–4175.
9. Nishiyama, Y.; Sugiyama, J.; Chanzy, H.; Langan, P. *J. Am. Chem. Soc.* **2003**, *125*, 14300–14306.
10. Nishiyama, Y.; Langan, P.; Chanzy, H. *J. Am. Chem. Soc.* **2002**, *124*, 9074–9082.
11. Langan, P.; Nishiyama, Y.; Chanzy, H. *Biomacromolecules* **2001**, *2*, 410–416.
12. Wada, M.; Heux, L.; Isogai, A.; Nishiyama, Y.; Chanzy, H.; Sugiyama, J. *Macromolecules* **2001**, *34*, 1237–1243.
13. Viëtor, R. J.; Newman, R. H.; Ha, M. A.; Apperley, D. C.; Jarvis, M. C. *Plant J.* **2002**, *30*, 721–731.
14. Murdock, C. C. *Phys. Rev.* **1930**, *35*, 8–23.
15. Ioyelovich, M. Ya. *Polym. Sci.* **1991**, *33*, 1670–1676.
16. Fink, H.-P.; Hofmann, D.; Philipp, B. *Cellulose* **1995**, *2*, 51–70.
17. Newman, R. H. *Solid-State Nucl. Magn. Reson.* **1999**, *15*, 21–29.
18. Kroon-Batenburg, L. M. J.; Kroon, J.; Northolt, M. G. *Polym. Commun.* **1986**, *27*, 290–292.
19. Koyama, M.; Sugiyama, J.; Itoh, T. *Cellulose* **1997**, *4*, 147–160.
20. Wada, M.; Okano, T.; Sugiyama, J. *Cellulose* **1997**, *4*, 221–232.
21. Broch, E. *Z. Phys. Chem.* **1927**, *127*, 446–454.
22. Swanson, H. E.; Tatge, E. In *Standard X-ray Diffraction Powder Patterns. National Bureau of Standards Circular 539*; US Government Printing Office: Washington, 1953; Vol. 1, pp 37–38.
23. Gjonnes, J.; Norman, N. *Acta Chem. Scand.* **1958**, *12*, 2028–2033.
24. Klug, H. P.; Alexander, L. E. *X-ray Diffraction Procedures for Polycrystalline and Amorphous Materials*; Wiley: New York, 1954; p 500.