Structure of cellulose microfibrils in primary cell-walls from collenchyma

Lynne H. Thomas, V. Trevor Forsyth, Adriana Šturcová, Craig J. Kennedy, Roland P. May, Clemens M. Altaner, David C. Apperley, Timothy J. Wess and Michael C. Jarvis

Supplementary Information

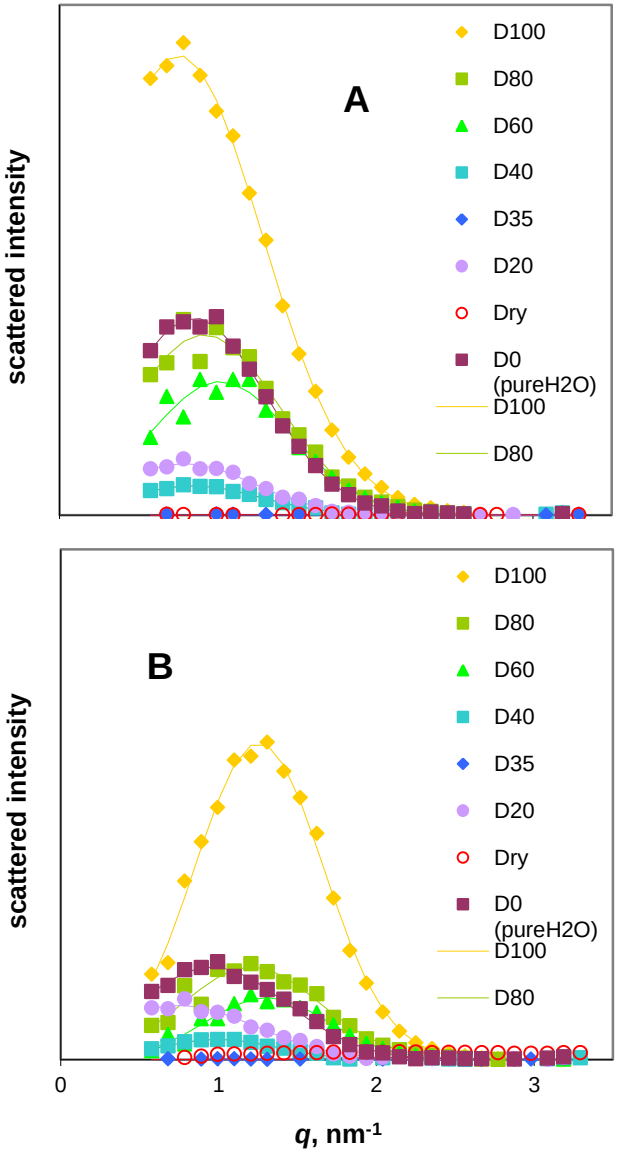
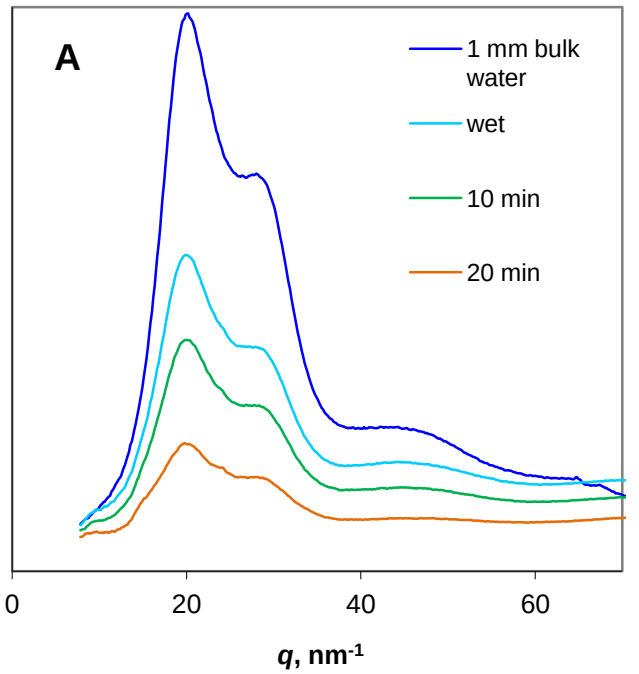


Fig. S1. Equatorial small-angle neutron scattering profiles background-corrected to remove the exponential component resulting from non-coherent scattering. The Gaussian component that remains is due to Bragg scattering from loosely arrayed microfibrils. **A:** celery collenchyma cell walls and **B:** isolated cellulose, saturated with D_2O (D100), 80%, 60%, 40%, 35% and 20% D_2O in H_2O (D80 to D20) and H_2O (D0)



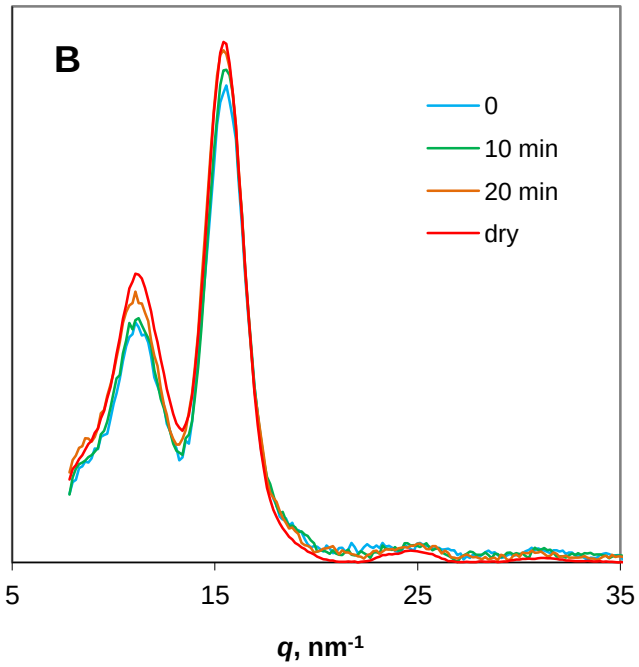


Fig. S2. Contribution of water to wide-angle X-ray scattering patterns from celery collenchyma cell walls. **A:** Radial, isotropic difference profiles (wet-dry) from cell walls saturated with water and after 10 min and 20 min air drying. The corresponding scattering profile from bulk liquid water is shown for comparison. **B:** Equatorial intensity profiles of the same samples after scaled subtraction of the isotropic scattering contribution from water, showing minor changes in the scattering contribution of cellulose consequent on hydration.

Methods

Collenchyma cell wall isolation

Individual strands of collenchyma tissue of celery (*Apium graveolens* L.) petioles were dissected from celery plants as described (Sturcova et al., 2004). The collenchyma strands were cut into pieces of approximately 5 cm length and placed into a 2 g/L aqueous Triton X100 solution for 60 min to remove plasma membrane proteins, while the solution was stirred occasionally and kept at a temperature of 4ºC - 6ºC. At the end of 60 minutes, nearly all of the Triton solution was removed and the celery collenchyma cell walls were rinsed thoroughly with deionised water. Each rinsed strand of celery collenchyma cell walls was placed carefully into a plastic Petri dish and allowed to dry at room temperature.

Cellulose isolation

Celery collenchyma cellulose was isolated in a manner initially similar to cell walls. After the short water rinse, the collenchyma strands were kept in a refrigerator for about 20 hrs and then rinsed thoroughly. Non-cellulosic polysaccharides present in collenchyma cell walls (mostly pectin) were removed by acid hydrolysis in 1 mol/l HCl at 100ºC for 60 min followed by thorough washing with deionised water. Each collenchyma cellulose strand placed individually into a plastic Petri dish and allowed to dry. The cellulose strands contained approximately 30 – 40% of the initial dry mass.

Small Angle Neutron Scattering (SANS)

SANS analysis was conducted on beamline D11 at the Institut Laue-Langevin (ILL), Grenoble, France.

D11 receives neutrons from the vertical cold source of the ILL high flux reactor, placing the detector in a low background environment. The incoming neutrons are monochromated, giving neutrons of a fixed wavelength within ± 9 %. Scattered neutrons were detected on a 64 x 64 cm CERCA ^{3}He multi-detector mounted on a moveable trolley within the evacuated detector tube, at a sample-to-detector distance of 4 m.

The q range covered at this experiment extended from 0.57 nm^{-1} to 3.3 nm^{-1} corresponding to real space values of 1.9-11 nm. Here q is defined as $(4\pi/\lambda)\sin(\theta)$, where λ is the neutron wavelength and θ is the scattering angle. Samples were placed in quartz sample cells with a 1 mm path length (Hellma, Germany). Images were taken from dry samples and samples soaked in fractions of D_2O of: 1, 0.8, 0.6, 0.4, 0.35 (the contrast match point of cellulose), 0.2 and 0. For each sample, the transmitted intensity was recorded and a background image taken.

Data recorded at D11 were analysed using the program GRASP (Charles Dewhurst, ILL). The data were corrected for variations in detector response and sample transmission, and a background image was subtracted, as described (Fernandes et al., 2011).

Wide-angle X-ray Scattering (WAXS).

The processing of the WAXS images was in general as described (Fernandes et al., 2011). After conversion into reciprocal space, background correction was carried out by two methods depending on whether the sample was dry or hydrated. For dry samples, at each value of q the azimuthal minimum of the scattered intensity was found, excluding the regions around the beamstop and on the fibre axis. The resulting radial profile was smoothed by a moving average over 0.3 nm^{-1} and assumed to be circularly symmetric. For dry samples a dual-exponential function was fitted to the minima before subtraction. For hydrated samples, the circularly symmetric background constructed as above was found to fit closely to the scaled radial intensity profile of the scattering image measured for a 1 mm path length of distilled water (Fig. S2) and the fitted intensity from the liquid water image was used for background correction.

The radial profiles of the 1-10, 110, 200 and 400 reflections were each fitted with an asymmetric function of the form:

$$I = 0.399 I_o (1+f(q)) \exp(-0.5((q-q_o)/\sigma)^2/\sigma$$

where I_o is the maximum intensity located at $q = q_o$ and $f(q) = a(q-q_o)^2$ when $q < q_o$ but zero when $q > q_o$. This function is based on a Gaussian profile with asymmetry introduced by the term $f(q)$ allowing the contribution of the scattering component contributing the asymmetry to be subtracted from the total (2).

Instrumental broadening was measured experimentally using powdered (<10μm) lanthanum hexaboride. For each reflection the observed σ value of the symmetric component was corrected for instrumental broadening by $\sigma_{obs}^2 = (\sigma_{corr}^2 + \sigma_{instr}^2)^{0.5}$ and was used to calculate the full width at half maximum $F = 2.355\ \sigma_{corr}$., from which the Scherrer dimensions and disorder factors were determined as described (2).

Wide-angle Neutron Scattering (WANS).

Collenchyma cell wall samples, carefully aligned and packed together into a sheet of dimensions 15 mm x 10 mm x 1 mm, were adjusted in hydration/deuteration level and encapsulated in aluminium foil as described previously for SANS (Fernandes et al., 2011).

WANS analysis was conducted on beamline D19 at the Institut Laue-Langevin (ILL), Grenoble, France. Beamline D19 has a four-circle diffractometer with a cylindrical detector consisting of a 256 x 640 array of gas-filled cells giving an aperture 30º vertically x 120º horizontally. The neutron wavelength was 2.418A and the sample-to-detector distance, taken to the electrode plane in each cell at the equator, was 756 mm. The response for each cell of the detector was calibrated using the isotropic incoherent neutron scattering from a vanadium rod, and blank-corrected using an empty aluminium foil container.

The absorption coefficient of each sample along the beam axis was measured in a separate experiment on beamline D22, and corresponded well to calculated absorption coefficients

based on the elemental composition. Absorption factors at all angles within the aperture of the detector were then calculated using in-house software based on the integrated path length through the sample, which was assumed to have cuboidal geometry and was wider than the neutron beam. The fibre axis was tilted such that the full widths of the 001, 002, 003 and 004 reflections were collected. In-house software was then used to remap the data into reciprocal space and to join together the component images of the diffraction pattern. The combined images were exported into Fit2D, where radial intensity profiles integrated over 10^{o} in azimuth were calculated in the equatorial and meridional directions.

Fernandes AN, Thomas LH, Altaner CM, Callow P, Forsyth VT, Apperley DC, Kennedy CJ, Jarvis MC (2011) Nanostructure of cellulose microfibrils in spruce wood. Proceedings of the National Academy of Sciences of the United States of America **108:** E1195-E1203

Sturcova A, His I, Apperley DC, Sugiyama J, Jarvis MC (2004) Structural details of crystalline cellulose from higher plants. Biomacromolecules **5:** 1333-1339