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Structure of cellulose microfibrils in mature cotton fibres

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ABSTRACT

The structure of cellulose microfibrils in mature cotton fibres from three varieties – *Gossypium hirsutum, G. barbadense* and *G. arboreum* – has been investigated by a multi-technique approach combining small angle scattering techniques with spectroscopy and diffraction. Cellulose microfibrils present a Iβ-rich crystalline structure with limited surface disorder. Small angle scattering (SAXS and SANS) data have been successfully fitted using a core-shell model and the results obtained indicate that the cellulose microfibrils, formed by the association of 2–3 elementary fibrils, are composed of a ca. 2 nm impermeable crystalline core, surrounded by a partially hydrated paracrystalline shell, with overall cross-sections of ca. 3.6–4.7 nm. Different low levels of cell wall matrix components have a strong impact on the microfibril architecture and enable moisture penetration upon hydration. Furthermore, the higher amounts of non-cellulosic components in *G. barbadense* result in a less dense packing of cellulose microfibrils and increased solvent accessibility.

1. Introduction

The plant cell wall (CW) is the structural component which encloses plant cells, providing protection against diverse stresses and offering support by resisting the action of turgor pressure, i.e. preventing over-expansion of cells by the uptake of water. The structure of plant CWs is complex but is commonly depicted as a system in which a semi-crystalline component, i.e. cellulose microfibrils, is embedded in an amorphous matrix of polysaccharides, glycoproteins and, in the case of secondary cell walls, lignin. The cellulose microfibrils act as the load-bearing component (Baskin, 2005; Geitmann, 2010), providing structural integrity, whereas the matrix components are able to tune the properties of plant tissues according to their specific requirements (Burton, Gidley & Fincher, 2010; Geitmann, 2010).

Due to the sequential character of its biosynthesis process (Brown, 1996; Delmer & Amor, 1995), cellulose is characterised by a hierarchical assembly of structural features (Martínez-Sanz, Gidley & Gilbert, 2015a; Martínez-Sanz, Mikkelsen, Flanagan, Gidley & Gilbert, 2016b). Cellulose molecular chains, consisting of glucose units connected by β-1-4-linkages, are typically arranged into larger structures known as cellulose microfibrils. These microfibrils contain distinct domains with differing levels of cellulose chain ordering: (i) crystalline (i.e. highly ordered chains), (ii) paracrystalline (i.e. regions with loose molecular packing or some degree of crystal distortion) and (iii) non-crystalline (i.e. regions without regular packing of cellulose chains) domains. Additionally, the presence of hydroxyl groups on the surface of the microfibrils leads to the creation of cellulose-cellulose and cellulose-water interactions by means of a strong hydrogen bonded network, resulting in larger structures which are referred to as "bundles", "macrofibrils" or "ribbons". The presence of CW matrix components may alter this complex cellulose architecture in different ways and at distinct structural levels, depending on the interaction mechanism of each component with cellulose. Although the investigation of the plant CW structure is of interest from a scientific and industrial perspective, research within this field has been challenging due to the inherent difficulty of studying these multicomponent

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systems, together with the significant heterogeneity of structures found within different plant species and tissues (Burton, Gidley & Fincher, 2010). Furthermore, elucidating the structural role of individual matrix polysaccharides is particularly complicated due to the ability of plants to adapt to modifications in their cell wall composition (Park & Cosgrove, 2012). The deconstruction approach typically applied to successively extract CW components is of limited relevance, since it is unclear to what extent the chemical and/or enzymatic treatments applied may affect the structure of the cellulosic network.

In this context, cotton represents an excellent model system to investigate the structure of cellulose in plant CWs (Haigler, Betancur, Stiff & Tuttle, 2012; Lee et al., 2015) since it consists of a single-cell system with a cellulose content (ca. 90–95 wt.% in mature cotton fibres) much higher than that typically found in plant CWs. Cotton fibres develop along five different stages known as (i) initiation, (ii) elongation, (iii) transition, (iv) secondary cell wall (SCW) synthesis and (v) maturation (Haigler, Betancur, Stiff & Tuttle, 2012). At the initiation stage, cotton fibres arise from the epidermal cell on the ovule surface. During the elongation phase, the fibres undergo expansion via primary cell wall (PCW) deposition (Haigler, Betancur, Stiff & Tuttle, 2012). Through the transition phase, the cellulose synthesis rate increases abruptly (Meinert & Delmer, 1977) and the first SCW layer is deposited. Subsequently, SCW thickening takes place via deposition of several layers of nearly pure cellulose. At the final maturation stage, cell death processes occur and the cotton boll opens. This phase is also characterised by fibre dehydration, leading to flattening and twisting and producing mature cotton fibres which typically present a kidney-shaped cross-section.

Cotton is the world's most important resource of renewable textile fibres. Amongst the *Gossypium* genus, the species *G. hirsutum, G. barbadense*, *G. arboreum* and *G. herbaceum* have been domesticated for fibre production (Hernandez-Gomez et al., 2015). While *G. hirsutum* accounts for the majority of the cotton fibre production due to its high yield and wide environmental adaptability, *G. barbadense* presents superior fibre quality (i.e. longer, finer and stronger fibres) (Avci, Pattathil, Singh, Brown, Hahn & Haigler, 2013; Li et al., 2016). In terms of industrial applicability, ensuring fibre quality is crucial to increase productivity and to manufacture textile products with the desired properties. Thus, knowledge of the structural and compositional changes undergone by different cotton varieties through their different developmental stages is essential to establish appropriate strategies to improve fibre quality parameters.

The investigation of the plant CW structure has been typically addressed by using microscopy and spectroscopy techniques, which require sample preparation processes that may induce structural changes. As an alternative, small angle X-ray and neutron scattering (SAXS and SANS) techniques offer the possibility of characterising partially hydrated native plant CW materials, while covering a size range from one to hundreds of nm. The great potential of these techniques has already been applied to construct a multi-scale model for the hierarchical architecture of cellulose in bacterially-sourced systems (Lopez-Sanchez et al., 2016; Martínez-Sanz, Gidley & Gilbert, 2016a; Martínez-Sanzanz, Lopez-Sanchez, Gidley & Gilbert, 2015b; Martínez-Sanz et al., 2016b; Martínez-Sanz et al., 2016c). However, their application to plant CW materials is still very scarce and, to date, has been limited to the characterisation of CWs from spruce wood (Fernandes et al., 2011; Jakob et al., 1995), dicots (Thomas, Forsyth, Martel, Grillo, Altaner & Jarvis, 2014) and celery collenchyma (Kennedy, Šturcová, Jarvis & Wess, 2007a; Kennedy et al., 2007b; Thomas et al., 2013). Although these studies have provided meaningful insights into the structure of cellulose in plant CWs, the relative abundance of matrix components in the native materials has precluded the establishment of the effect of each component on the structure of cellulose. In the present work, we exploit the potential of SAXS and SANS, combined with complementary diffraction and spectroscopy techniques, to construct a model for the structure of cellulose microfibrils in a relatively pure cellulose plant CW system, namely, cotton. Such an approach is expected to open up the possibility for further studies on the structure of cellulose in diverse plant CW systems. In the particular case of cotton, this would allow investigation of the evolution of cellulose structure during the various developmental phases, and aid in the enhancement of fibre quality for industrial applications.

2. Materials and methods

2.1. Preparation of mature cotton samples

Cotton plants were grown under glasshouse conditions in Canberra, Australia (35°18′27″S 149°07′27.9″E, 580m) using natural light and with a temperature minimum set to 20 °C (10:00 pm–6:00 am) and maximum set to 32 °C (6:00 am–10:00 pm). Plants were watered daily via an automatic watering system and fertilised with slow-release commercial fertiliser just before flowering and then, as required, until bolls matured for harvesting. Unless stated otherwise, fibre was removed from seeds by hand for further analysis.

2.2. Polysaccharide composition

Polysaccharide composition was deduced from monosaccharide linkage analysis of mature cotton fibre alcohol insoluble residue as described by Pettolino, Walsh, Fincher & Bacic (2012).

2.3. Scanning electron microscopy (SEM)

Mature fibre was teased away from a mid-point along the seed and threaded through a hole 0.8 mm in diameter in a 1 mm-thick stainless steel slide. The fibre was cut at the slide surface on both sides with a sharp blade and the upper cut surface imaged by SEM in backscatter mode on a Zeiss EVO LS15 environmental scanning electron microscope.

2.4. Small angle neutron scattering (SANS)

SANS measurements were performed on the 40 m QUOKKA instrument at the OPAL reactor (Gilbert, Schulz & Noakes, 2006), as described previously (Martínez-Sanz et al., 2016a). Three configurations were used to cover a q range of 0.004–0.8 $Å^{-1}$ where q is the magnitude of the scattering vector defined as $q = \frac{4\pi}{\lambda} \sin\theta$ and 2θ is the scattering angle. These configurations were: (i) source-to-sample distance (SSD) = 20.2 m, sample-to-detector distance (SDD) = 20.1 m, (ii) SSD = 3.9 m, SSD = 4.0 m and (iii) SSD = 10.0 m, SDD = 1.4 m using a wavelength, λ, of 5.0 Å of 10% resolution and with source and sample aperture diameters of 50 mm and 10 mm, respectively. Native cotton samples were placed in 1 mm path length cells with demountable quartz windows, which were then filled with the required solvent (H_2O, D_2O or different H_2O/D_2O mixtures). To maximize D/H exchange, prior to the SANS measurements, the native cotton fibres were soaked in D_2O or H_2O/D_2O mixtures with an approximate sample/solvent ratio of 0.1 g/100 mL. The samples were initially soaked for 24 h and, subsequently, an additional exchange step with fresh solvent was carried out for at least a further 24 h. Native cotton samples were also studied by placing them between two quartz windows within a demountable cell with adjustable path length. SANS data were reduced using NCNR SANS reduction macros (Kline, 2006) modified for the QUOKKA instrument, using the Igor software package with data corrected for detector sensitivity, empty cell scattering and transmission and transformed onto an absolute scale using an