Polymer 124 (2017) 1–11

Contents lists available at ScienceDirect

Polymer

journal homepage: www.elsevier.com/locate/polymer

# Multi-scale characterisation of deuterated cellulose composite hydrogels reveals evidence for different interaction mechanisms with arabinoxylan, mixed-linkage glucan and xyloglucan

Marta Martínez-Sanz [a, b, 1], Deirdre Mikkelsen [b], Bernadine M. Flanagan [b], Michael J. Gidley [b], Elliot P. Gilbert [a, c, *]

[a] Australian Centre for Neutron Scattering, Australian Nuclear Science and Technology Organisation, Locked Bag 2001, Kirrawee DC, New South Wales, 2232, Australia
[b] ARC Centre of Excellence in Plant Cell Walls, Centre for Nutrition and Food Sciences, Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, St. Lucia, Brisbane, QLD 4072, Australia
[c] Australian Institute for Bioengineering and Nanotechnology, Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, St. Lucia, Brisbane, QLD 4072, Australia

ARTICLE INFO

*Article history:*
Received 26 May 2017
Received in revised form
11 July 2017
Accepted 13 July 2017
Available online 14 July 2017

*Keywords:*
Small angle scattering
Neutron scattering
Deuteration
Cellulose
Mixed-linkage glucans
*Komagataeibacter xylinus*

ABSTRACT

The interactions of cellulose with other major plant cell wall polysaccharides - arabinoxylan (AX), xyloglucan (XG) and mixed linkage glucans (MLG) - have been investigated by characterising the architecture of composite deuterated cellulose hydrogels by means of SAXS and SANS, combined with XRD, NMR and microscopy. The results indicate that cellulose-AX interactions, limited to the ribbons' surface, take place via a non-specific adsorption mechanism. In contrast, XG and MLG interact specifically with cellulose, forming two different fractions: (i) interfibrillar domains interacting with the cellulose microfibrils and (ii) surface domains, responsible for the cross-linking of ribbons. XG co-crystallises with cellulose, promoting the formation of $I_\beta$-richer microfibrils and forming intercalated amorphous regions. On the other hand, MLG interacts with cellulose forming a paracrystalline coating layer. This structural role of XG and MLG in preventing microfibril aggregation may help explain their key function in the cell expansion process of growing plant tissues.

© 2017 Elsevier Ltd. All rights reserved.

## 1. Introduction

Plant cell walls (PCWs) are extremely complex systems in which cellulose microfibrils, the main load-bearing component, are embedded in an amorphous matrix of polysaccharides and glycoproteins. While the cellulose microfibrils represent the main building block in most PCWs, the specific properties of each plant tissue are strongly determined by the matrix cell wall composition and the nature of the interactions established between the different cell wall constituents. Xyloglucan (XG) and pectins are the major non-cellulosic polysaccharides found in the primary cell walls from dicotyledonous plants and non-commelinoid monocotyledonous plants; however, they are present only in very small amounts in the cell walls from Poaceae (cereals/grasses) and related commelinoid monocotyledons [1–6]. In the latter, arabinoxylans (AX) and (1 → 3) (1 → 4)-β-D-glucans, i.e. mixed linkage glucans (MLG), constitute the predominant matrix polysaccharides (Yokoyama & Nishitani, 2004). For each specific plant species, the composition and structure differ depending on additional factors such as growth phase, cell type and cell position [7,8].

Knowledge of the interaction mechanisms between cellulose and matrix polysaccharides is essential to unequivocally determine the structural role of non-cellulosic polysaccharides and understand the effect of the aforementioned factors on the structure of PCWs. For instance, the ability of certain fractions of XG and pectins to establish direct molecular interactions with cellulose microfibrils has been supported by several studies [7–16]; however, these two polysaccharides are thought to interact with cellulose by means of different mechanisms. XG appears to interfere with the cellulose

* Corresponding author. Australian Centre for Neutron Scattering, Australian Nuclear Science and Technology Organisation, Locked Bag 2001, Kirrawee DC, New South Wales, 2232, Australia.
*E-mail address:* elliot.gilbert@ansto.gov.au (E.P. Gilbert).
[1] Present address: Food Quality and Preservation Department, IATA-CSIC, Avda. Agustin Escardino 7, 46980 Paterna, Valencia, Spain.

http://dx.doi.org/10.1016/j.polymer.2017.07.036
0032-3861/© 2017 Elsevier Ltd. All rights reserved.

crystallisation process during its biosynthesis, leading to the formation of less crystalline, smaller and $I_\beta$-richer cellulose microfibrils [12–14]. On the other hand, pectins have a minor effect on the cellulose crystallinity, although they have been reported to affect the cellulose network assembly, inducing a strong densification effect [10,11]. MLG has been commonly considered as the analogue for XG in the primary cell walls of cereals/grasses, tethering cellulose microfibrils and forming a load-bearing network [1,17,18]. Nevertheless, a recent *in vitro* study questioned this hypothesis and suggested that MLG is rather found as a layer coating the surface of cellulose microfibrils [19]. MLG is abundant in the endosperm of grains, where it is thought to act as a storage carbohydrate [1,20]. In addition, MLG may play an important role in the cell expansion of certain tissues, where its concentration increases with the cell enlargement process and decreases sharply after cessation of growth [7,21,22]. However, in other tissues MLG remains within the primary cell wall after the cell expansion process has been completed [8,23,24]. Thus, it is evident that more detailed studies are required to elucidate the interaction mechanism of MLG with cellulose, providing valuable insights on the structural role of this PCW polysaccharide.

Although the composition of diverse PCWs has been extensively studied and is well reported, the limited information available on the specific mechanisms through which matrix polysaccharides interact with each other and/or with cellulose during the biosynthesis process is mainly due to the inherent complexity of *in planta* studies. Genetic studies are one of the most widely used methods to investigate the structural role of PCW polysaccharides. However, since plants are able to adapt to certain variations in their cell wall composition (Park & Cosgrove, 2012), it is not possible to ascertain how they compensate for modifications in their genetic code through pleiotropic effects. Another commonly applied strategy consists of the sequential extraction of matrix constituents by means of chemical and/or enzymatic treatments. In this case, the uncertainty of how the cellulose network and/or additional components are affected by each extraction process also precludes the drawing of definitive conclusions. In this context, the synthesis of cellulose hydrogels by means of bacterial fermentation has been demonstrated to serve as an efficient tool to investigate the structure and interaction mechanisms of cellulose with matrix PCW components [9,13,25–29]. This approach enables the incorporation of selected PCW components into the bacterial culture medium to determine their effect on the structure of the synthesized composite hydrogels without interference from additional components. The application of multiple techniques based on the combination of small angle X-ray and neutron scattering (SAXS and SANS) with complementary spectroscopy, diffraction and microscopy has been recently shown to represent an excellent approach to characterise the structure of native hydrogels, covering the whole size range relevant to the hierarchical architecture of cellulose (i.e. from the molecular arrangement into crystalline, paracrystalline and amorphous domains to form the individual cellulose microfibrils, to the association of several microfibrils to form cellulose ribbons) [13,30]. In addition to the minimal sample preparation required for SAXS and SANS, and thus avoiding structural alterations induced by drying, the possibility of enhancing the scattering length density (SLD) contrast in SANS experiments by selective deuterium labelling has been shown to provide a significant advantage for the application and validation of complex models to fit the experimental data and extract valuable structural information [30,31].

In this study, we explore the structural effect of three major PCW matrix polysaccharides, namely AX, XG and MLG, on the hierarchical architecture of partially deuterated cellulose hydrogels. The strategic combination of the selected techniques has enabled the identification of structural modifications induced by the presence of these PCW polysaccharides at the different relevant size ranges in the hierarchically-assembled cellulose network and the elucidation of their different interaction mechanisms with cellulose. This will not only provide meaningful insights in research areas such as plant biology, the development and of bio-based and biodegradable polymeric materials and the optimisation of the processes used to synthesise biofuels from lignocellulosic biomass [32–34], but also insight into the use of these materials for medical and tissue-engineering applications [35].

## 2. Materials and methods

### 2.1. Production of pure cellulose and composite hydrogels

Deuterated cellulose, as well as associated composite PCW hydrogels were prepared as described by Martínez-Sanz et al. [30], and also following the detailed methodology described by Mikkelsen and co-workers [27,36]. All polysaccharide solutions were made at 1.0% (w/v) and, by adding them to the Hestrin and Schramm basal medium, as required, resulted in growth media containing a final polysaccharide concentration of respectively 0.5% (w/v) medium viscosity (22 cSt) wheat AX (lot 40302b), medium viscosity (28 cSt) barley MLG (lot 90802), or tamarind seed XG (lot 100403) (Megazyme International Ireland, County Wicklow, Ireland). Incubations were performed as previously reported at 30 °C for 48 h under static conditions, thereafter harvested, washed and stored in 0.02% (w/v) sodium azide solution at 4 °C, until used for experiments. The wet weights of all samples were recorded as previously described by Martínez-Sanz et al. [30].

### 2.2. Compositional analysis

AX and XG polymer incorporation into the D-CH-AX and D-CH-XG composite hydrogels was estimated as previously described [30]. Briefly, the air-dried samples were cut with a scalpel, hydrolysed with sulphuric acid, reduced and acetylated. Gas chromatography-mass spectrometry (GC-MS) was subsequently used to identify and quantify the alditol acetates of the monosaccharides.

The mixed-linkage β-D-glucan assay kit (Megazyme International Ireland Ltd, County Wicklow, Ireland) was used according to the manufacturer's protocol to assess the amount of MLG in the D-CH-MLG composite hydrogel.

### 2.3. Scanning electron microscopy (SEM)

Field emission SEM (FESEM) was used to visualise the microarchitectures of the fully hydrated D-CH and composite hydrogels as previously described by Martínez-Sanz et al. [30].

### 2.4. Small angle neutron scattering (SANS)

SANS measurements were performed on the 40 m QUOKKA instrument at the OPAL reactor [37]. Three configurations were used to cover a q range of ca. 0.004–0.8 $Å^{-1}$, where $q$ is the magnitude of the scattering vector defined as $q = \frac{4\pi}{\lambda}\sin\theta$, λ is the wavelength in Å and $2\theta$ 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 = 1.3 m, with 10% wavelength resolution and λ = 5.0 Å. The source and sample aperture diameters were 50 mm and 10 mm, respectively. Native D-CH and composite hydrogels were studied by placing the samples in sealed 1 mm path length cells with demountable quartz windows and filling the cells with the required solvent ($H_2O$, $D_2O$ or different $H_2O/D_2O$

Download English Version:

https://daneshyari.com/en/article/5177881

Download Persian Version:

https://daneshyari.com/article/5177881

Daneshyari.com