International Journal of Biological Macromolecules 102 (2017) 111–118

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Fibrillar assembly of bacterial cellulose in the presence of wood-based hemicelluloses

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ARTICLE INFO

Article history:
Received 18 October 2016
Received in revised form 31 March 2017
Accepted 3 April 2017
Available online 6 April 2017

Keywords:
Bacterial cellulose
Hemicellulose
Hierarchical structure
Small-angle scattering

ABSTRACT

Composite materials mimicking the plant cell wall structure were made by culturing cellulose-producing bacteria together with secondary-wall hemicelluloses from wood. The effects of spruce galactoglucomannan (GGM) and beech xylan on the nanoscale morphology of bacterial cellulose were studied in the original, hydrated state with small-angle X-ray scattering (SAXS). The SAXS intensities were fitted with a model covering multiple levels of the hierarchical structure. Additional information on the structure of dried samples was obtained using scanning and transmission electron microscopy and infra-red spectroscopy. Both hemicelluloses induced a partial conversion of the cellulose crystal structure from I_α to I_β and a reduction of the cross-sectional dimensions of the cellulose microfibrils, thereby affecting also their packing into bundles. The differences were more pronounced in samples with xylan instead of GGM, and they became more significant with higher hemicellulose concentrations.

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1. Introduction

The plant cell wall is a complicated, hierarchical composite structure, where different components have distinct functions. In the secondary cell wall of plants, cellulose molecules form rigid microfibrils, that are bundled into larger structures together with other polymers, most of all hemicelluloses and lignin. Even though the nanoscale structure of the plant cell wall and its formation have been subjects of research over a long time, many essential features like the exact morphology of the cellulose microfibril (CMF) and factors governing their lateral packing into bundles after the cellulose biosynthesis still remain to be elucidated [1,2]. A deeper understanding of these structural features and their formation would help us to better utilize the abundant, renewable biomass resources of our planet and to develop tailored cellulose materials for various applications [3–7].

Structure formation of the plant cell wall can be mimicked by a model system consisting of bacterial cellulose (BC) and hemicelluloses. The production of such composite material is fairly simple: a cellulose-producing bacterial strain is incubated in a culture medium together with the desired hemicellulose and as a result, a composite structure is formed by self-assembly of the different components. Previous experimental works utilizing this approach have shown that some hemicelluloses interfere with the formation of CMFs and aggregates thereof, causing various changes in their structure [8–21]. Typically, the incubation together with hemicelluloses resulted in looser CMF bundles, smaller crystal size, and changing of the dominant crystalline allomorph from cellulose I_α to I_β.

However, except for two individual works dealing mostly with changes in the cellulose crystals [11,16], the previous studies used either primary-wall hemicelluloses, like xyloglucan, or commercially available versions of the secondary-wall hemicelluloses. These hemicelluloses often originate from non-wood sources and have a lower molecular weight and fewer side groups as compared to their native counter parts. Another major shortage of most previous works is that they studied the materials only after drying. It is well known, that the morphology of fibrillar cellulosic materials is highly sensitive to aggregation and other changes caused by drying, especially at the level of interest in the current case. These effects cannot be fully prevented even if advanced drying techniques like freeze-substitution are used.

Small-angle scattering techniques provide excellent tools for studying the average nanoscale structure of BC in its original, hydrated state. However, various ways of interpreting the scattering patterns exist in the literature. The small-angle X-ray and neutron scattering (SAXS/SANS) intensities of BC and BC-containing materials have been fitted with Guinier laws [22–25], power laws [25–27], as well as analytical expressions for shapes varying from concentric cylinders [12,13,28] to platelets [29]. Most of the previously applied models do not take into account the hierarchical

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http://dx.doi.org/10.1016/j.ijbiomac.2017.04.010
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structure of bacterial cellulose, but rather describe the system in a simplified way, either as single CMFs or their bundles. This is an issue related also to the few past studies applying small-angle scattering on biomimetic BC–hemicellulose composites [12,13,22].

One option to model small-angle scattering from the hierarchical structure of BC is to use the unified exponential/ power-law model proposed by Beaucage [30,31], which has recently been applied to various other fibrillar cellulosic materials [32–37]. In this model, each structural level contributes to the scattering in two ways: an exponential term based on the Guinier law and corresponding to the overall outer dimensions of a particle on low values of the scattering vector q, and a power-law term describing the surface or inner structure of the particle on higher q values. The contributions of different structural levels partly overlap each other and the power-law regimes are limited at both ends. The model does not assume any particular shape for the structures and can therefore be used to approximately describe rod-like systems also [30].

In this work, the effects of native, wood-based secondary-wall hemicelluloses on the assembly of CMFs and their bundles were studied by utilizing cellulose-producing bacteria. The nanoscale structure of the BC–hemicellulose composites was characterized with synchrotron SAXS in their original, hydrated state. Additional information from dried samples was obtained with electron microscopy and infra-red (IR) spectroscopy.

2. Experimental

2.1. Preparation of BC–hemicellulose composites

Komagataeibacter sucrofermentans (JCM 9730, formerly known as *Gluconacetobacter sucrofermentans*) was grown in an artificial, glycerol-based culture medium [29] with varying concentrations (0.05%, 0.1%, 0.5%) of two wood-based hemicelluloses: acetylated galactoglucomannan (GGM), molar mass 60 kg/mol, extracted from spruce (*Picea abies*) and precipitated in ethanol [38], and acetyl-4-*O*-methylglucuronoxylan (xylan), molar mass 24 kg/mol, DMSO-extracted from beech (*Fagus sylvatica*) [39]. Details of the hemicelluloses are presented in Table S1 of the Supplementary material. A control sample was prepared without adding hemicelluloses. In order to induce preferred orientation in the fibrillar network [40], the bacteria were cultured inside of oxygen-permeable silicone tubes (AS ONE 6-586-05; inner/outer diameter 2/3 mm, length 10 cm) sealed at both ends. After incubating for about one week at 28 °C, the formed BC–hemicellulose tubes were washed adapting the procedures of He et al. [29]: soaking in water, heating up to 90 °C, soaking two times 1–2 h in 0.1 M NaOH at room temperature, and washing with water several times. The samples were stored in Milli-Q water at 4 °C.

2.2. SAXS measurements

The nanostructure of the BC–hemicellulose tubes was characterized with synchrotron SAXS at the BL40B2 beamline of SPring-8 in Hyogo, Japan. The wavelength of the X-rays was $\lambda = 0.100$ nm and sample-to-detector distance 4.15 m, allowing a q range from 0.03 nm^{-1} to 2 nm^{-1} to be measured (scattering vector $q = 4\pi \sin(\theta)/\lambda$ with 2θ being the scattering angle). The scattered radiation was detected with an R-AXIS VII image-plate area detector (Rigaku). Approximately 1-cm pieces of the tubular, gel-like samples in aqueous solution were placed in glass capillaries (diameter 2 mm, dry-matter content roughly 1%) oriented perpendicular to the incident X-ray beam. SAXS data from freeze-dried samples in air and freeze-dried and rewetted samples in water were also collected.

The SAXS patterns were corrected for background noise and normalized by measurement time and incident flux. The corresponding background for each sample (water in capillary or empty sample holder) was multiplied by a transmission factor to correct for beam attenuation in the sample and subtracted from the intensities of the samples. Each sample except the freeze-dried and rewetted was measured at several positions and the intensities were averaged.

2.3. SAXS data analysis

The SAXS intensities were fitted using the unified exponential/power-law model [30,31] with three levels of structures ($i = 1, 2, 3$):

$$\begin{aligned} I_{fit}(q) \;&= G_1 \exp\left(-\frac{q^2 R_{g,1}^2}{3}\right) + B_1 \left(\frac{\left[\operatorname{erf}\left(qk_1 R_{g,1}/\sqrt{6}\right)\right]^3}{q}\right)^{P_1} \\ &+ G_2 \exp\left(-\frac{q^2 R_{g,2}^2}{3}\right) \\ &+ B_2 \exp\left(-\frac{q^2 R_{g,1}^2}{3}\right) \left(\frac{\left[\operatorname{erf}\left(qk_2 R_{g,2}/\sqrt{6}\right)\right]^3}{q}\right)^{P_2} \\ &+ B_3 \exp\left(-\frac{q^2 R_{g,2}^2}{3}\right) \frac{1}{q^{P_3}} + C \end{aligned} \tag{1}$$

In Eq. (1), $R_{g,i}$ is the radius of gyration of particles belonging to the structural level i, P_i is the power-law exponent corresponding to the inner/surface structure of these particles, G_i and B_i are the Guinier and power-law prefactors, respectively, and the constant C corresponds to a q-independent background. The smallest detectable structures are here defined as structural level $i = 1$ and the largest as $i = 3$. The value of the fudge factor k_i is 1 for $P_i > 3$ and 1.06 for $1.5 < P_i < 3$, as based on empirical observations [31]. Using the model of Eq. (1) requires that the spatial organization of the scattering particles is too weak to produce any observable correlation or diffraction peak. Instead, the shoulder features observed in the SAXS intensities are assumed to arise from the outer dimensions of the scattering particles.

The fitting was done with the freely-available IRENA package [41] for IGOR Pro software (WaveMetrics). The data was rebinned to 200 points and the square root errors for each data point were calculated by the programme. A fixed value of 4 was used for P_1, whereas all other parameters were allowed to vary freely. Error estimates for the fitting parameters were determined by the fitting software. Further calculations were carried out using MATLAB R2012a (MathWorks).

2.4. Scanning electron microscopy

Field-emission scanning electron microscopy (SEM) imaging was performed with a JEOL JSM-6700F microscope operated at 1.5 kV, using the lower secondary electron (LEI) detector. Prior to imaging, the samples were freeze-dried, placed on two-sided conductive tape, and coated with platinum (JEOL JFC-1600; 10 mA, 90 s).

2.5. Transmission electron microscopy and image analysis

Samples for transmission electron microscopy (TEM) were prepared by incubating the bacteria for 2–4 h in the culture medium containing 0.1% hemicellulose (plus control), after which a small volume of the culture medium was deposited on a glow-discharged, carbon-coated copper mesh, and incubated for further 15 min. The samples were washed twice with 40 mM HEPES-NaOH (pH 7.4), 150 mM NaCl buffer, negatively stained using 2% uranyl acetate, and

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