European Polymer Journal 104 (2018) 177–183

Contents lists available at ScienceDirect

European Polymer Journal

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

Biomimetic composites of deuterated bacterial cellulose and hemicelluloses studied with small-angle neutron scattering

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ARTICLE INFO

Keywords:
Bacterial cellulose
Hemicellulose
Deuteration
Small-angle neutron scattering

ABSTRACT

Composites mimicking the plant-cell wall structure were prepared by synthesizing deuterated bacterial cellulose in D-based culture medium including hemicelluloses from wood. The partial intracrystalline deuteration of bacterial cellulose was demonstrated by FT-IR spectra, which also showed that the hemicelluloses modified the cellulose crystal structure in both the water-accessible and water-inaccessible parts of the fibrils. The hierarchical wet-state structure of the composites was characterized using small-angle neutron scattering (SANS) and the cross-sectional fibril dimensions were compared to scanning electron microscopy images of freeze-dried samples and earlier small-angle X-ray scattering results from hydrogenated samples. The SANS data of deuterated bacterial cellulose/hemicellulose composites revealed a decrease of the microfibril cross-section and looser packing of the microfibrils into wider bundles in the presence of hemicelluloses.

1. Introduction

Cellulose is an extremely abundant polymer of glucose, typically found in plant-cell walls but also produced by some bacteria [1,2]. In the secondary cell wall of plants such as wood, cellulose forms a strongly hierarchical structure based on fibrillar semi-crystalline cellulose microfibrils embedded in a matrix composed of lignin and a group of branched heteropolysaccharides called hemicelluloses. The assembly of cellulose molecules into microfibrils and larger fibrillar structures immediately or soon after their biosynthesis is believed to be affected by the simultaneous incorporation of hemicelluloses in the fibril aggregates. This idea is mainly based on observations that adding water-soluble hemicelluloses to the culture medium of cellulose-producing bacteria affects the formation and morphology of the fibrillar bacterial cellulose (BC) structures [3–6]. However, in most earlier studies applying this approach, the hydrogel-like cellulose/hemicellulose composites were dried for morphological characterization, which may not give a correct picture on the original wet-state structure.

Small-angle neutron and X-ray scattering (SANS and SAXS) are powerful methods for characterizing the hierarchical structure of BC and other cellulosic materials under their original, wet state. They have been previously applied to various types of BCs [7–10], including also biomimetic composites of BC and hemicelluloses [11–15]. As a recent improvement for SANS, methods to synthesize partly or even fully deuterated BC by culturing properly adapted bacterial strains in ^{2}H-based culture medium have been introduced [16–18]. This allows an increase of the scattering length density (SLD) contrast between cellulose fibrils and the surrounding solution from 2×10^{10} cm^{-2} or 5×10^{10} cm^{-2} for hydrogenated BC (H-BC) in H_2O or D_2O, respectively, up to around 7×10^{10} cm^{-2} for deuterated BC (D-BC) in H_2O [17,19]. At the same time, the crystallinity, crystal size, and fibril morphology of the BC remain largely unaffected [16,18,20]. Moreover, deuteration can be used for contrast variation in systems containing multiple components with different SLDs. Controlled deuteration of BC has already been utilized in some studies applying SANS [13,20,21] and other neutron scattering techniques [22–25], but it was not yet used to understand the assembly of BC fibrils in the presence of wood-based hemicelluloses.

The objective of this work was to utilize the improved SLD contrast of deuterated BC in 100% H_2O solution to address the effects of two wood-based hemicelluloses on the fibrillar assembly of BC. The structure of the composites was characterized using SANS, FT-IR spectroscopy and scanning electron microscopy (SEM), and the results were compared to those from similar H-based composites studied earlier with SAXS and other methods [14].

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https://doi.org/10.1016/j.eurpolymj.2018.05.015
Received 29 December 2017; Received in revised form 6 April 2018; Accepted 13 May 2018

2. Experimental

2.1. Preparation of D-BC/hemicellulose composites

D_8-Glycerol (99%) and D_2O (99.9%) were purchased from Cambridge Isotope Laboratories. Acetylated galactoglucomannan (GGM) (molar mass 60 kg/mol), extracted from spruce (*Picea abies*) and precipitated in ethanol [26], was received as a gift from Prof. Stefan Willför, Åbo Akademi University. Acetyl-4-*O*-methylglucuronoxylan (xylan) (molar mass 24 kg/mol), DMSO-extracted from beech (*Fagus sylvatica*) [27], was received as a gift from Prof. Bodo Saake, University of Hamburg.

Gluconacetobacter sucrofermentans (strain JCM 9730) was adapted to grow in D_2O-based Schramm-Hestrin (SH) medium [28] on an agar plate by gradually increasing the D_2O/H_2O ratio from 0% to 100% with steps of 20 percentage points, incubating the bacteria for 4–7 days at 30 °C between the steps. A deuterated version of the artificial culture medium developed by He et al. [17] was prepared by mixing 80 mL 50% (w/w) D_8-glycerol (in D_2O), 4 mL 10% sodium lactate (in H_2O), 10 mL vitamin solution (in H_2O) and 10 mL mineral salts solution (in H_2O) with 896 mL basal salts solution (in D_2O). The pH was adjusted to about 5.5 by adding 1 µL of 1-M H_2SO_4 per 1 mL of the culture medium. The vitamin solution consisted of 200 mg/L inositol, 40 mg/L nicotinic acid, 40 mg/L pyridoxal hydrochloride, 40 mg/L thiamine hydrochloride, 20 mg/L calcium D-panthothenate, 20 mg/L riboflavin, 20 mg/L *p*-amino benzoic acid, 0.2 mg/L D-biotin and 0.2 mg/L folic acid in H_2O. The mineral salts solution consisted of 360 mg/L $FeSO_4{\cdot}7H_2O$, 1270 mg/L $CaCl_2$, 242 mg/L $Na_2MoO_4{\cdot}2H_2O$, 173 mg/L $ZnSO_4{\cdot}7H_2O$, 139 mg/L $MnSO_4{\cdot}5H_2O$ and 5 mg/L $CuSO_4{\cdot}5H_2O$ in H_2O. The basal salts solution consisted of 2 g/L $(NH_4)_2SO_4$, 3.3 g/L KH_2PO_4, 3.3 g/L $Na_2HPO_4{\cdot}12H_2O$, 1.8 g/L $MgSO_4{\cdot}7H_2O$ and 3.3 mg/L boric acid in D_2O. The sodium lactate solution, vitamin solution, and basal salts solution were sterilized by filtering with a 0.2 µm cellulose acetate filter (ADVANTEC, DISMIC-13), whereas the mineral salts solution was autoclaved at 121 °C.

The D_2O-adapted bacteria were first transferred from the SH-agar plate and precultured in 4 mL of the D_8-glycerol-based medium on a small petri dish. After 10 days of incubation at 28 °C, the formed preculture pellicle was soaked and briefly vortexed in fresh D_8-glycerol-based culture medium to extract the bacteria. The new culture medium containing bacteria from the preculture was divided into three portions: without added hemicelluloses, with 0.5% GGM and with 0.5% xylan. The cultures were inserted in silicone tubes with inner diameter 2 mm (AS ONE 6-586-05) and length 10 cm, sealed from both ends and incubated for 2 weeks in horizontal position at 28 °C.

When the cultures were finished, the tubular D-BC/hemicellulose composites were washed by soaking in H_2O for 1 h, brought to boiling in H_2O, soaked two times 2 h in 0.1-M NaOH and washed with H_2O several times. Slow magnetic stirring was applied during all steps.

2.2. Characterization of D-BC/hemicellulose composites

Attenuated total reflectance (ATR) FT-IR spectra were measured with a Perkin Elmer Frontier FT-IR spectrometer. Freeze-dried samples were pressed on the probe crystal and 16 scans with 4-cm^{-1} resolution on the spectral range from 400 to 4000 cm^{-1} were averaged. The spectra were converted to absorbance and baseline-corrected, after which a constant background (signal around 1800 cm^{-1}) was subtracted and the spectra were normalized to the absorbance at 1110 cm^{-1}.

Field-emission SEM imaging was done using a JEOL JSM-7800F Prime microscope with operation voltage 1.5 kV and lower secondary electron detector. The freeze-dried samples were gently pressed on double-sided conductive tape and coated with platinum (JEOL JFC-1600; 10 mA, 90 s) prior to imaging. The width of over 300 fibrils per sample was measured manually using the Gwyddion 2.48 software [29] from images taken at 50,000-times magnification.

SANS measurements [30] were done at the D11 instrument of Institut Laue–Langevin (ILL). Sample-to-detector distances of 1.5 m, 8 m and 34 m with neutron wavelength $\lambda = 0.6$ nm ($\Delta\lambda/\lambda = 0.09$) were used to cover a total q range from 0.02 to 4 nm^{-1}, with the magnitude of the scattering vector being $q = 4\pi\sin\theta/\lambda$ and scattering angle 2θ. The tubular samples were folded with the tube axis mostly horizontal and placed together with H_2O inside of quartz glass cells with 2 mm optical path. Corrections to the raw data, azimuthal integration, and merging of data from different detector distances were done using LAMP, the Large Array Manipulation Program [31]. The SANS intensities were fitted with the unified exponential/power-law model [32] with contributions from three levels of hierarchy (i = 1,2,3 in increasing order of structure size):

$$I(q) = G_1\exp\left(-\frac{q^2R_{g,1}^2}{3}\right) + G_2\exp\left(-\frac{q^2R_{g,2}^2}{3}\right) + B_2\exp\left(-\frac{q^2R_{g,1}^2}{3}\right)\left(\frac{[\mathrm{erf}(qR_{g,2}/\sqrt{6})]^3}{q}\right)^{P_2} + B_3\exp\left(-\frac{q^2R_{g,2}^2}{3}\right)\frac{1}{q^{P_3}} + C, \quad (1)$$

where $R_{g,i}$ is the radius of gyration of particles at structural level i, P_i is the power-law exponent corresponding to their inner/surface structure, and G_i, B_i and C are constants. The power-law term of level $i = 1$ was not included in the fit, because it tended to converge towards zero for all samples. The fitting was done using the IRENA package [33] for IGOR Pro software (WaveMetrics). Uncertainty of the fitting parameters was evaluated in the software with the chi-squared range target fixed to 1.01.

3. Results and discussion

3.1. Deuteration and molecular structure of the composites

The ATR FT-IR spectra of all deuterated BC samples and a hydrogenated BC sample for comparison are presented in Fig. 1. The spectrum of pure deuterated BC ("D-BC") as well as the spectra of D-BC with GGM ("D-BC + GGM") and xylan ("D-BC + xylan") showed two absorbance regions originating from deuterated molecular groups: the O–D stretching bands at 2600–2400 cm^{-1} and the C–D stretching bands around 2100 cm^{-1}. The spectrum of a corresponding sample without deuteration ("H-BC") contained only the O–H and C–H stretching bands at 3500–3300 cm^{-1} and around 2900 cm^{-1}, respectively, which appeared weaker in the deuterated samples. As the deuterated samples were washed and stored in H_2O, it is assumed that at least in the D-BC sample the signals in the O–H stretching region correspond to OH groups that were accessible to water, whereas the bands around 2500 cm^{-1} originated from inaccessible or possibly intracrystalline OD groups. On the other hand, the simultaneous presence of both C–H and C–D stretching bands in the D-BC sample indicates that the deuteration of cellulose was not complete, which could be caused by the presence of a small amount of hydrogenated compounds in the culture medium. The same observations apply to the samples with hemicelluloses, even though some signal especially in the O–H and C–H stretching regions might also be due to the presence of hemicelluloses. Based on visual inspection of the spectra, the H/D ratio appeared roughly similar in all deuterated samples and a clearly detectable amount of both H and D were bound to C and O. However, due to various factors affecting the relative signal intensities in ATR FT-IR spectra, including the sample–crystal contact and orientation [34], the data is not well suited for accurate determination of the H/D ratio.

Despite the limitations of the method, examination of the closely located FT-IR bands characteristic of the cellulose I_α and I_β crystalline allomorphs (Fig. 1, insets) is possible and yields some interesting information. Firstly, the D-BC sample exhibited the signals from both