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Preparation and enzymatic hydrolysis of nanoparticles made from single xyloglucan polysaccharide chain

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ABSTRACT

In this work, polysaccharide nanoparticles based on tamarind seeds xyloglucan are prepared, analyzed in term of characteristic sizes and morphology, and degraded by the action of a glycoside-hydrolase. Obtained in an aqueous $NaNO_2$ solution (0.1 M), these unaggregated nanoparticles have a characteristic diameter of ca. 60 nm (DLS, AFM and TEM measures). They are not compact, but highly swollen and look like hyperbranched and dendrimer-like (soft sphere model) structures. This observation is coherent with the native structure of the xyloglucan macromolecules which are themselves branched. The enzymatic hydrolysis by cellulase of *Trichoderma reesei* of the xyloglucan nanoparticles is investigated. In particular, the apparent mass molecular weight drastically decreases meaning that the xyloglucan nanoparticles are effectively fully hydrolyzed by the endo-β-(1,4)-glucanase. Furthermore, we observe that the enzyme has to uncoil the nanoparticles before cutting the β-(1→4) bonds and digesting the xyloglucan.

1. Introduction

Polysaccharides are abundant natural polymers and can come out of several resources: animal, algae, microorganisms or plants. They are an inexhaustible source of advanced and sustainable materials exhibiting usually non-toxicity, biodegradability, biocompatibility and sometimes biorecognition properties. In the field of the preparation of polysaccharide-based nanoparticles (Schatz & Lecommandoux, 2010; Sundar, Kundu, & Kundu, 2010), the drug delivery devices (Liu, Jiao, Wang, Zhou, & Ziyong, 2008) are particularly relevant and can be prepared by emulsification, desolvation or coacervation (Langer et al., 2003) depending on the desired targeted application.

Xyloglucan is a hemicellulose polysaccharide that is found in the primary cell wall of higher plants (Fry et al., 1993; Hayashi, 1989) and can be present in seeds like tamarind where it represents 50% (w/w) (Shankaracharya, 1998) of the storing energetic resources. The chemical structure of tamarind seed xyloglucan consists of a cellulose-like main chain of β-(1→4)-linked D-glucosyl residues that is regularly substituted at C-6 with α-D-xylosyl and α-D-galactosyl-(1→2)-α-D-xylosyl residues (Nishinari, Takemasa, Zhang, & Takahashi, 2007; York, Vanhalbeek, Darvill, & Albersheim, 1993). The availability of the xyloglucan (in term of quantity and purity) allowed the development of a large number of commercial, industrial and pharmacological applications (Gidley et al., 1991; Picout, Ross-Murphy, Errington, & Harding, 2003; Rao & Srivastava, 1973). It is widely used as thickeners, gelling agents or stabilizers in food industry in Japan and other Asian countries. Xyloglucan gels are used for drug delivery (Coviello, Matricardi, & Alhaique, 2006; Miyazaki et al., 1998), and recently xyloglucan-based nanoparticles were prepared to encapsulate an anti-cancer drug (Cao et al., 2010).

Nevertheless high molecular weight xyloglucan chains present some limitations such as uncontrolled water solubility and a high polydispersity which originate from their ability to form large aggregates via hydrogen bonding (Picout et al., 2003). Nevertheless, the partial degradation of the xyloglucan can be achieved by its digestion with endo-β-(1,4)-glucanase which cuts the backbone at unsubstituted D-glucose positions (Fanutti, Gidley, & Reid, 1993).

In the present paper, we prepared xyloglucan nanoparticles, which were characterized by dynamic light scattering (DLS), gel permeation chromatography (GPC), and transmission electron microscopy (TEM). In fact, DLS represents a powerful method for the characterization of biomacromolecules in solution and for monitoring their degradation (Lima, Soldi, & Borsali, 2009;

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Wu, Zhou, & Wang, 1995) while AFM has been successfully used to image polysaccharides morphology or to probe the interaction forces with other biomacromolecules like polysaccharides or proteins (Liu, Fu, Zhu, Li, & Zhan, 2009; Lubambo et al., 2009; Murai, Hokonohara, Takagi, & Kawai, 2009). We then investigated the enzymatic hydrolysis of these nanoparticles by a commercial endo-β-(1,4)-glucanase from *Trichoderma reesei* using DLS and AFM as techniques of analysis. This study is mandatory for further use of xyloglucane based nanoparticles in drug delivery applications where it is important to know the response mechanism of the nanoparticle to a possible enzymatic hydrolysis.

2. Materials

Xyloglucan from *Tamarindus indicia* was supplied by Saiguru Food Gum Manufacturer Pvt. Ltd. (Mumbai, Maharashtra, India). The xyloglucan was dissolved in water, put to boil and filtered to remove insoluble compounds. Then it was lyophilized. The powder was stored before further use.

The apparent mass molecular weight of xyloglucan was determined to be 4×10^5 g/mol by gel permeation chromatography using a Waters GPCV Alliance 2000 chromatograph from Wyatt (USA) equipped with three online detectors (a differential refractometer, a viscometric detector and a light scattering detector MALLS) and a Shodex OHpak SB-805 HQ column (8–300 mm; exclusion limit, 4×10^6). The solution was injected at a concentration of 1×10^{-3} g/L in 0.02% NaN_3 aqueous solution containing $NaNO_3$ (0.1 M) and EDTA (10 mM) as eluant and solvent.

Cellulase (C2730 from *T. reesei* ATCC 26921, ≥700 units/g) was purchased from Sigma and used without any purification.

3. Methods

3.1. Dynamic light scattering (DLS) of xyloglucan

3.1.1. Sample preparation

Solutions for DLS measurement (1 g/L) were prepared with sodium nitrite $NaNO_2$ (0.1 M) made with ultrapure Milli-Q water. The solutions were stirred overnight, then heated at 80 °C for 2 h. To eliminate dust and other large particles, all samples were filtered through 0.45 and 0.22 μm cellulose acetate filter (Millipore) prior to measurements.

3.1.2. DLS measurements

These measurements were performed using an ALV laser goniometer, which consists of a cylindrical 33 mW HeNe linear polarized laser with a 632.8 nm wavelength and an ALV-5000/EPP multiple-tau digital correlator with a 125 ns initial sampling time. The samples were kept at a constant temperature of 25 °C throughout the experiments. The accessible scattering angle of this equipment ranges from 40 up to 120°. All samples were systematically studied at 90°. The solutions were put in ordinary glass cells. The minimum sample volume required for an experiment was ca. 1 mL. The data acquisition was done with the ALV-correlator control software and the counting time for each sample was 300 s. The relaxation time distributions $A(q,t)$ were obtained using CONTIN analysis of the autocorrelation function $C(q,t)$.

3.2. Atomic force microscopy (AFM)

3.2.1. Sample preparation

The substrate used in this work was hydrophilic silicon with an approximate dimension 1 cm × 1 cm. Perfectly cleaned silicon was obtained by chemical treatment of the substrate with piranha solution H_2SO_4/H_2O_2 mixture (70:30, v/v) at 80 °C for one hour, followed by intensive rinsing with water and dry blowing by N_2 (Fritzen-Garcia et al., 2009). 20 μL of the xyloglucan suspension (the same suspension as the one used for DLS measurements) was deposited on the hydrophilic silicon surface, and then dried overnight at room temperature in a dessicator.

3.2.2. AFM imaging

AFM imaging was performed at room temperature using a Pico plus (Molecular Imaging, Phoenix, USA) commercial instrument using a 100 μm × 100 μm piezoelectric scanner. 512 × 512 data points were acquired. Images were obtained in tapping mode, using silicon tips with a spring constant of 48 N/m and a resonance frequency of approximately 190 kHz (VISTA probes). Data treatment (i.e. height measurements after baseline correction only) and presentation were realized with the help of Gwyddion software.

3.3. Transmission electron microscopy (TEM)

The morphology of the xyloglucan nanoparticles was examined using a Philips CM200 transmission electron microscope (FEI Company, Hillsboro, USA) operating at 120 kV. The images were recorded on Kodak SO163 films. Drops of the xyloglucan suspension (1 g/L) in nitrite sodium (0.1 M) were deposited onto glow discharged hydrophilic carbon-coated copper grids and negatively stained with 2% (w/v) uranyl acetate; the liquid in excess was blotted with filter paper.

3.4. Enzymatic hydrolysis

3.4.1. Capillary viscometry

The hydrolytic action was monitored by measuring the changes in specific viscosity as a function of incubation time using an automatized capillary viscosimeter (Schott 531 10). The enzymatic degradation experiments were carried out at 25 °C with 15 mL aqueous solution of xyloglucan prepared in 0.1 M $NaNO_2$ (1 g/L). The commercial cellulase was diluted 10 times and 10 μL were added into the medium. The viscosity was measured every 5 min during hydrolysis and the specific viscosity was calculated as $(T - T_0)/T_0$ where T_0 is the flow time measured for the solvent and T is the flow time of the reaction mixture containing the enzyme.

3.4.2. Gel permeation chromatography (GPC)

The molar mass distributions for the intact and the enzymatically hydrolyzed xyloglucan samples were analyzed by SEC/MALS/RI. The intact xyloglucan and the hydrolysates were dissolved at a concentration of 1 g/L. All samples were filtered through a 0.22 μm cellulose acetate filter (Millipore) before the analysis. The data from the detectors were processed using ASTRA software (ASTRA V 5.3.4.14 from Technology Corp.). Refractive index increment dn/dc = 0.153 mL/g used for the calculations was determined using a refractometer Optilab rEX (Wyatt Technology). Double injections were made for all samples.

3.4.3. Dynamic light scattering (DLS)

For enzymatic hydrolysis, 1 μL of cellulase from *T. reesei* (diluted 10 times in 0.1 M $NaNO_2$) was injected into 1 mL of xyloglucan suspension at a concentration of 1 g/L. The scattering intensity and the hydrodynamic radius were detected in real-time during the enzymatic hydrolysis. The reaction was followed for a period of 4 h.

3.4.4. Atomic force microscopy (AFM)

For AFM experiments, xyloglucan nanoparticles dried onto silicon wafer were incubated with the cellulase for various periods of time at room temperature. The surface was then rinsed by water