Carbohydrate Polymers 129 (2015) 216–223

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Transient and quasi-permanent networks in xyloglucan solutions

Rilton Alves de Freitas[a,b,*], Vivian C. Spier[b], Maria Rita Sierakowski[b], Taco Nicolai[a], Lazhar Benyahia[a], Christophe Chassenieux[a]

[a] LUNAM Université, Université du Maine, CNRS UMR 6283, IMMM, Avenue Olivier Messiaen, 72085 LE MANS Cedex 9, France
[b] BioPol, Chemistry Department, Federal University of Paraná, 81531-980 Curitiba, PR, Brazil

ARTICLE INFO

Article history:
Received 6 February 2015
Received in revised form 4 April 2015
Accepted 27 April 2015
Available online 8 May 2015

Keywords:
Xyloglucan
Gel
Viscosity
Transient network

ABSTRACT

Viscoelastic properties of aqueous solutions of xyloglucan extracted from *Hymenaea courbaril* seeds (Jatobá gum) were investigated by rheology over a wide range of concentrations and temperatures. The polymer was characterized in dilute solutions by light scattering measurements and size exclusion chromatography. Xyloglucan formed, in semi-dilute solutions (C 0.3 wt%), a transient network with cross-links characterized by a broad distribution of lifetimes, independent of the temperature and concentration. Progressively, at higher temperatures (>60 °C), a second much weaker quasi-permanent network was formed and attributed to the exchange of intra- to inter-chain bonds. The stiffness of the second network increased with decreasing temperature, but it could be easily broken by applying a relatively weak shear stress and is readily reversible on re-heating, and partially reversible on resting at 20 °C.

1. Introduction

Xyloglucan is a polysaccharide that has a structural function in the cell wall of plants and works as a reserve of carbohydrate in seeds of some species of dicotiledoneae. The xyloglucan found in seeds has principally the monosaccharides D-glucose, D-xylose and D-galactose, usually at molar proportions of 4:3:1, respectively. The backbone of xyloglucan is composed by units of D-glucopyranose (G), linked β-(1 → 4), with substitutions at glucose O-6 by α-D-xylopyranose (X), and some xyloses can be also substituted at O-2 by β-D-galactopyanose (L) (Hayashi, 1989; Reid, 1985).

Tamarind xyloglucan (also denominated Tamarind gum) isolated from *Tamarindus indica L.* seeds is used as a thickening agent in food and pharmaceutical industries. Xyloglucan can also be isolated from seeds of *Hymenaea courbaril* as an alternative source and is called Jatobá gum. The structure of xyloglucan chains from these two different sources is close, except that Tamarind gum only contains sequences with two or three consecutive X units, whereas Jatobá gum also contains sequences with four consecutive X units (Fig. 1) (Buckeridge et al., 1997; Freitas et al., 2005).

Because of the use of xyloglucan as a thickening agent several authors have studied the viscoelastic properties of xyloglucan aqueous solutions as a function of the concentration (Khounvilay & Sittikijyothin, 2012; Martin, Freitas, Obayashi, & Sierakowski, 2003; Wang, Ellis, Ross-Murphy, & Burchard, 1997). The viscosity of semi-dilute solutions, i.e. above the overlap concentration of xyloglucan, was found to increase strongly with increasing concentration and shear thinning was observed for the more viscous solutions. The frequency dependence of the shear moduli was typical for viscoelastic polymer solutions, with a solid-like behavior at high oscillation frequencies and liquid-like behavior at low frequencies. Wang et al. (1997) suggested that the rheology of semi-dilute xyloglucan solutions was caused by entanglement of the polymeric chains.

Sims et al. (1998) compared the viscosity of xyloglucan originating from different species of plants, including Nicotiana, Apple pomace and Tamarind. They reported that the viscosity at a given polymer concentration strongly depended on the origin of the xyloglucan, due to differences in the molar mass. They noted that the viscosity decreased with increasing temperature, but did not depend significantly on the pH.

Though the viscosity of aqueous xyloglucan solutions has been investigated in some detail, the concentration and temperature dependence of the frequency dependent shear moduli has not yet been studied systematically. Therefore it has not been possible to fully understand the dynamic mechanical properties of xyloglucan solutions.

* Corresponding author at: BioPol, Chemistry Department, Federal University of Paraná, 81531-980 Curitiba, PR, Brazil. Tel.: +55 41 3361 3260; fax: +55 41 3361 3186.
E-mail addresses: rilton@ufpr.br, rilton@quimica.ufpr.br, rilton@pq.cnpq.br (R.A. de Freitas).

http://dx.doi.org/10.1016/j.carbpol.2015.04.066
0144-8617/

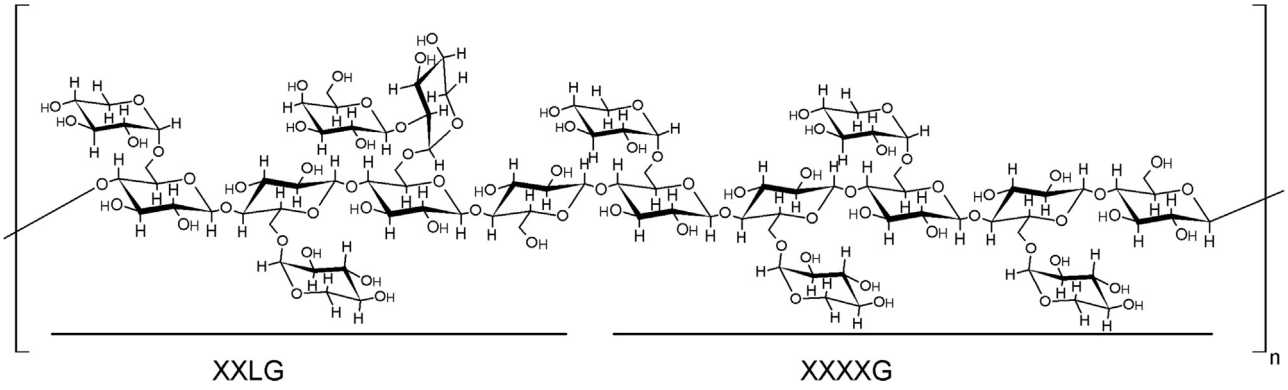

Fig. 1. Schematic representation of two oligosaccharide segments in the xyloglucan chain, the backbone is a β-(1 → 4) D-glucopyranose (G), with substitutions at O-6 by α-D-xylopyranose (X), and at O-2 by β-D-galactopyanose (L). For the oligosaccharide nomenclature used see Fry (1989).

The aim of the investigation presented here was to characterize and elucidate the rheology of xyloglucan in aqueous solution. We studied solutions of xyloglucan from *H. courbaril* seeds, over a wide range of temperatures (10–90 °C) and concentrations (0.1–5 wt%) using continuous and oscillatory shear rheology. The structure of the polymers in dilute solution was characterized by light-scattering and size exclusion chromatography. The viscoelastic behavior of the xyloglucan solutions will be discussed in comparison with similar behavior reported for guar gum (Wientjes, Duits, Jongschaap, & Mellema, 2000) and hydroxylpropyl methyl cellulose (HPMC) (Shahin, Nicolai, Benyahia, Tassin, & Chassenieux, 2013).

2. Materials and methods

2.1. Material

Seeds of *H. courbaril* were obtained from Project "matas nativas", Itatinga, São Paulo, Brazil. The xyloglucan was obtained by aqueous extraction at 25 °C of pooled and milled seeds. Each viscous extract was centrifuged at 10,000 × *g* for 20 min at 20 °C, and the supernatant was collected. The xyloglucan gum was obtained after its precipitation with two volumes of 96% ethanol, washed with 96% ethanol and with one volume of acetone (Freitas et al., 2005)

The carbohydrate content was determined according Freitas et al. (2005), as 90% of total carbohydrates, 2% of proteins and 8% moisture.

2.2. Characterization

After extraction and chemical characterization, the xyloglucan oligosaccharide composition was determined using the enzyme cellulase (*endo*-1,4-β-D-glucanase/EGII), from *Trichoderma longibrachiatum*, that was purchased from Megazyme (Bray Co., Wicklow, Ireland) and was used without further purification.

Quantification of oligosaccharides obtained after enzymatic digestion was done by high-performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC) analysis using a Thermo Scientific ICS-5000 system with a Carbopack PA-100 column (Thermo Scientific Dionex, Sunnyvale, CA, USA), ED gold electrode and an amperometric pulse detector (PAD). The eluent used was NaOH 88 mmol L^{-1} with a gradient of NaOAc 1 mol L^{-1} from 7 to 15% (v/v), flow rate of 0.9 mL min^{-1} at 30 °C. The data were treated with the Chromeleon 7 program (Thermo Scientific Dionex, Sunnyvale, CA, USA), used to identify and quantify the oligosaccharides composition.

The macromolecular characterization of xyloglucan was also performed. Weight average molar mass (M_w) and dispersity ($Đ = M_w/M_n$) were determined by size exclusion chromatography (SEC) at room temperature with a Tosho G6000PW column. The light scattering detector was a Dawn EOT™ (Wyatt technology, Santa Barbara, CA, USA) and the refractive index was measured using a Shodex RI 71 (Showa Denko K.K., Tokyo, Japan). A volume of 100 μL of the 0.05 wt% xyloglucan solutions was injected using an automatic injection system (Autoinjector 234, Gilson, Middleton, WI, USA). The system was eluted with 0.1 mol L^{-1} $NaNO_3$ at pH 7, with a flow rate of 1 mL min^{-1}. The data were analyzed using the Software ASTRA 6.1.1.

Light scattering (LS) measurements were done at 20 °C over a range of scattering wave vectors ($6.39 \times 10^6 - 2.55 \times 10^7$ m^{-1}) using an ALV-CGS3 equipment (ALV-GmbdH Langen, Germany). From these experiments M_w, the *z*-average radius of gyration (R_g) and the second virial coefficient (A_2) were determined following standard methods. Briefly, samples were prepared at different concentrations (0.01, 0.0275, 0.05, 0.10 and 1.00 wt%) and filtered through 0.2 μm pore-size Anotop® filters. From the excess scattering intensity normalized by that of a toluene standard (I_r), M_w, R_g and A_2 were determined using the following expression (Brown, 1993; Nicolai, 2007):

$$\frac{KC}{I_r} = \frac{1}{M_w} + \frac{(R_g)^z}{3M_w}q^2 + 2A_2C$$

where K is an optical constant appropriate for vertically polarized incident light:

$$K = \frac{4\pi^2 n^2}{\lambda^4 N_a}\left(\frac{\partial n}{\partial C}\right)^2\left(\frac{n_s}{n}\right)^2\frac{1}{R_s}$$

with n is the refractive index of the solvent and n_s and R_s the refractive index and Rayleigh scattering of the toluene, respectively. N_a is Avogadro's number, and λ is the wavelength of the laser (632.8 nm). The refractive index increment of xyloglucan is $\partial n/\partial C = 0.113$ mL g^{-1} (Freitas, Martin, Paula, Feitosa, & Sierakowski, 2004).

3. Rheology

The xyloglucan powder was dissolved at 20 °C in ultrapure water (Millipore system, Millipore) to which 200 ppm of NaN_3 was added as a bacteriostatic agent. The xyloglucan solutions from 0.1 wt% to 5 wt% were kept at 20 °C for at least 48 h, in the solvent prior to analysis, and the pH of solutions was 6.8 in all experiments. All the concentrations are expressed in weight %.

Oscillatory and continuous shear experiments were done using a rheometer AR 2000 Advanced Rheometer, TA instruments (New Castle, DE, USA). In both cases a cone and plate geometry was used