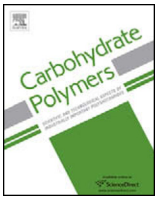
Carbohydrate Polymers 131 (2015) 392–398

Contents lists available at ScienceDirect

Carbohydrate Polymers

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



Novel thiol- amine- and amino acid functional xylan derivatives synthesized by thiol–ene reaction

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ARTICLE INFO

Article history:
Received 20 March 2015
Received in revised form 12 May 2015
Accepted 1 June 2015
Available online 8 June 2015

Keywords:
Xylan
Hydrogel
Polythiol
Thiomer
Thiol-ene
Click chemistry
Modification of polysaccharides

ABSTRACT

In the present work, novel thioether xylans were synthesized via a simple procedure using water as solvent. First, allyl groups were introduced on the backbone of xylan by etherification of allyl chloride in aqueous alkaline conditions at 40 °C, providing degree of substitution (DS) values up to 0.49. On the second step, the allyl groups were reacted with thioacetic acid, cysteamine hydrochloride or cysteine providing novel thiol-, amine- or amino acid functionalized xylans. The presented modular approach offers broad possibilities for developing new polysaccharide based materials. The thioacetic acid - ene reaction is reported for the first time for polysaccharide modification, yielding a protected thiol that can be stored at atmospheric conditions and can be deprotected by simple hydrolysis just prior to use, providing a versatile water soluble polythiol. The free thiol-groups were utilized for hydrogel formation through thiol–thiol oxidative coupling, allowing good control over the hydrogel shape, such as 3D hydrogel scaffolds and cross-linked foams. Further, the thiol-containing xylan was used to modify filter paper surface by a simple dipping method, which provides a novel and convenient way for introducing thiol-functionality on paper surface.

1. Introduction

Increasing environmental concern and need to decrease societal dependency on fossil based raw materials have motivated research towards diversifying products made from renewable sources. Polysaccharides can be found in vast amounts in nature, hemicellulose being the most abundant family of polysaccharides next to cellulose that can be found in most plant cell walls. Xylan, which is the main hemicellulose type in hardwood species, consists predominantly of D-xylopyranoside units connected by β-(1 → 4)-linkages along with acetyl-, glucuronic acid and species dependent side groups (Deutschmann & Dekker, 2012). Xylans and xylan derivatives have found applications in the paper and food industry as well as pharmaceutical applications (Ebringerová & Heinze, 2000; Ebringerová & Hromádková, 1999). It can be obtained from plant biomass, amongst other methods, by alkali extraction, which also hydrolyzes the acetyl groups rendering the backbone hydroxyl groups available for derivatization reactions (Ebringerová & Heinze, 2000; Glasser, Kaar, Jain, & Sealey, 2000). Examples of modifications targeting the hydroxyl groups of xylan include etherification using epoxides (Bigard et al., 2011; Ebringerová, Hromádková, Kacuráková, & Antal, 1994; Jain, Sjöstedt, & Glasser, 2001; Kataja-aho, Haavisto, Asikainen, Hyvärinen, & Vuori, 2011; Laine et al., 2013; Pahimanolis, Sorvari, Luong, & Seppälä, 2014; Pohjanlehto, Setälä, Kammiovirta, & Harlin, 2011; Ren, Sun, & Liu, 2007; Schwikal, Heinze, Ebringerová, & Petzold, 2006) and alkyl halides (Fang, Fowler, Tomkinson, & Hill, 2002; Petzold, Schwikal, & Heinze, 2006; Petzold, Günther, Kötteritzsch, & Heinze, 2008; Saghir, Iqbal, Koschella, & Heinze, 2009; Vincendon, 1998) in alkaline media and esterification reactions employing anhydrides (Buchanan et al., 2003; Fundador, Enomoto-Rogers, Takemura, & Iwata, 2012; Hansen & Plackett, 2011; Hettrich et al., 2006; Salam, Pawlak, Venditti, & El-tahlawy, 2011) or activated carboxylic acids (Daus & Heinze, 2010; Hesse, Liebert, & Heinze, 2006) and sulfating agents (Daus et al., 2011; Hettrich et al., 2006).

The thiol–ene reaction has gained much attention in chemical synthesis, popularized by click-chemistry concepts, inspired by the way molecular diversity is constructed in biological systems using rather simple building blocks (Hoyle & Bowman, 2010; Kolb, Finn, & Sharpless, 2001). The reaction can usually be performed under mild

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http://dx.doi.org/10.1016/j.carbpol.2015.06.007
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reaction conditions giving high conversion and selectivity, using water as solvent. In addition, no toxic metal catalysts are needed, making the use of thiol-ene chemistry tempting for the modification of polysaccharides. Despite being widely applied to synthetic polymers (Hoyle & Bowman, 2010; Lowe, 2010, 2014), the thiol-ene approach for polysaccharide modification has been limited. Previously, cellulose films having ene or thiol-functionalities were synthesized using silane chemistry (Huang, Li, & Gray, 2014; Tingaut, Hauert, & Zimmermann, 2011) or esterification and etherification (Nielsen, Eyley, Thielemans, & Aylott, 2010; Zhao, Hafrén, Deiana, & Córdova, 2010) and used for thiol-ene derivatization and cross-linking (Rosilo, Kontturi, Seitsonen, Kolehmainen, & Ikkala, 2013). In addition, reaction of pentenoic acid esters of hyaluronic acid and dextran with different thiols under UV light has been reported (Mergy, Fournier, Hachet, & Auzély-Velty, 2012).

Thiols and amines are valuable functional groups having high reactivity. They are useful for developing novel materials and in bio-inspired polymer synthesis (Le Neindre & Nicolaÿ, 2014). Although thiol containing polymers, thiomers (Bonengel & Bernkop-Schnürch, 2014), have been known for some time (Schwenker, Lifland, & Pacsu, 1962), the introduction of thiol groups is somewhat challenging due to their high reactivity leading to crosslinking or other side-reactions. We present herein a simple method for functionalizing xylan with thiols, amines and amino acids by combining traditional etherification and thiol-ene reactions.

2. Experimental

2.1. Materials

Birch wood xylan was purchased from Sisco Research Laboratories Pvt. Ltd. (Sugar composition: Xylose 93.1%, 4-O-Methylglucuronic acid 4.1%, Glucose 1.6%, Galactose 0.7%, Rhamnose 0.2%, Arabinose 0.1%, Glucuronic acid 0.1%, Galactouronic acid 0.1% determined by acid methanolysis (Willför et al., 2009); degree of acetylation less than 4% determined with ^{1}H-NMR, Mn = 11700 g/mol, PDI = 2.02 determined by GPC against PEG standards). Allyl chloride (98%), cysteamine HCl (97.0%), L-cysteine (97%), 5,5′-dithio-bis(2-nitrobenzoic acid) (99%), hydrogen peroxide (30 wt% solution in water), NaOH (99%), potassium peroxydisulfate (KPS) (99.0%) and thioacetic acid (96%) were purchased from Sigma-Aldrich. Acetic acid (99.8%) was from Merck and HCl solution 4 M AVS Titrinorm was obtained from VWR International. All chemicals were used as received.

2.2. Introducing allyl groups to the backbone of xylan

The allyl functionalization of xylan was done as follows:

To a xylan suspension (10.00 g in 50 ml of water), 20.0 ml of 5 M NaOH solution was added and the mixture was stirred at 40 °C for 30 min. To the obtained solution, 5.00 ml of allyl chloride (61.7 mmol) was added, the reaction vessel was closed with a silicone cap and the reaction was allowed to proceed for 24 h. The mixture was then neutralized with acetic acid and the product was collected by precipitation in 300 ml of ethanol, followed by purification by two more dissolution-precipitation steps and lyophilized to obtain 8.23 g of water soluble allylated xylan (M_n = 15200, PDI = 2.62), having a DS = 0.49 calculated from ^{1}H-NMR integral ratio of the allyl-proton (5.72–6.00 ppm) to the C1 proton (4.29–4.57 ppm) of the xylose unit. The obtained xylan derivative was stored in dark at room temperature.

2.3. Thiol-ene functionalization of xylan

The reactions with thiols (thioacetic acid, cysteamine hydrochloride, cysteine) were done as follows:

To a solution of allylated xylan (0.100 g in 1.00 ml of water), the thiol (thioacetic acid 0–0.130 g, cysteamine HCl 0.048–0.097 g or cysteine 0.052–0.102 g) was added and allowed to dissolve. In the case of thioacetic acid, 0–0.214 ml of 4 M HCl solution was also added to the mixture. Nitrogen gas was bubbled in to the solution for 5 min and the reaction was started by the addition of freshly prepared solution of potassium peroxydisulfate (6.1 mg or 12.2 mg in 0.220 ml of water). The reaction was allowed to take place for 30 or 60 min at 22 °C under vigorous stirring. When thioacetic acid was used as the reagent, a precipitate was formed within 5 min from the addition of the initiator, whereas clear solutions were maintained with cysteamine and cysteine. The product was precipitated in ethanol and purified by dissolution-precipitation steps until neutrality and lyophilized to obtain typically 0.080–0.090 g of modified xylan. The multi-gram scale experiment with thioacetic acid was done as above using 60 times multiplication of weights and volumes.

2.4. Hydrogel preparation using thiol–thiol coupling

The hydrogel scaffold was produced as follows: acetylthiopropyl xylan (0.30 g) and 3.00 ml of 1 M NaOH was stirred under a nitrogen atmosphere for 2 h. To the obtained solution, 3.00 ml of deoxygenated distilled water was added and the mixture was stirred for additional 1 h. A 3D printed polycaprolactone template (approx. 1.5 × 1.5 × 0.5 cm^3) was then immersed in the solution in order to completely fill the voids of the template. The crosslinking was initiated by transferring the template in a phosphate buffer (pH = 7.4) containing 0.4 M H_2O_2 for 15 min and then allowed to stand in pure buffer for one week at 7 °C. The polycaprolactone template was then removed by dissolution in dichloromethane for 4 days, changing fresh solvent periodically, leaving the hydrogel scaffold which was stored in pure buffer solution at 7 °C.

The hydrogel foams were prepared as follows: acetylthiopropyl xylan (0.20 g) and 2.00 ml of 1 M NaOH solution was stirred under a nitrogen atmosphere for 2 h. The obtained clear solution was then exposed to air and rapidly diluted to 2 wt% with distilled water (8.00 ml) and the pH was adjusted to 7 with 86 μl of glacial acetic acid. The solution was then foamed in a 250 ml beaker using a magnetic stirring bar (5 cm × 0.8 cm) with a 1400 rpm stirring speed for 10 min. The obtained foam was then dried at 70 °C overnight.

Surface modification of filter paper: acetylthiopropyl xylan (0.50 g) and 5.00 ml of 1 M NaOH was stirred under a nitrogen atmosphere for 2 h. To the obtained solution 5 ml of deoxygenated distilled water was added and the solution was diluted with 40 ml of 0.13 M acetic acid solution. The obtained solution was used for surface modification of filter paper (Whatman® Grade 3 cotton 98% α-cellulose) by dipping and then drying at 50 °C. To check reversibility of xylan adsorption, filter paper samples were also washed with distilled water to remove unattached xylan. Reference modifications were also done using unmodified xylan. The thiol content of modified filter paper samples were tested using the Ellman's method as described below.

2.5. Characterization

NMR spectra were recorded on a Bruker Avance-III 400 spectrometer in deuterium oxide (D_2O) or dimethyl sulfoxide (d-DMSO), using standard ^{1}H-, ^{13}C-, DEPT-, COSY- and HSQC pulse sequences. A relaxation delay of 40 s was used for quantitative ^{1}H-experiments. Carbon-13 cross-polarization/magic angle spinning nuclear magnetic resonance (^{13}C-CP/MAS NMR) spectra were recorded with a double resonance 4 mm probe. Samples were spun in zirconia rotors using a spinning rate of 10 kHz. A proton excitation pulse of 2.95 μs and a CP contact time of 2.0 ms were used applying a linear ascending variable amplitude ramp, the