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Targeted allylation and propargylation of galactose-containing polysaccharides in water

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ABSTRACT

Galactose units of spruce galactoglucomannan (GGM), guar galactomannan (GM), and tamarind (galacto)xyloglucan (XG) were selectively allylated. Firstly aldehyde functionalities were formed at the C-6 position via enzymatic oxidation by galactose oxidase. The formed aldehydes were further derivatized by an indium mediated Barbier–Grignard type reaction, resulting in the formation of homoallylic alcohols. In addition to allylic halides, the same reaction procedure was also applicable for GGM, when using propargyl bromide as halide. All reaction steps were done in water, thus the polysaccharides were modified in a one-pot reaction. The formation of the allylated, or propargylated, product was identified by MALDI-TOF–MS. All polysaccharide products were isolated and further characterized by GC–MS or NMR spectroscopy. By this chemo-enzymatic process, we have demonstrated a novel method for derivatization of GGM and other galactose-containing polysaccharides. The derivatized polysaccharides are potential platforms for further functionalizations.

1. Introduction

Naturally occurring non-cellulosic polysaccharides, such as wood and plant hemicelluloses, are becoming increasingly interesting as starting materials in the search for environmentally sustainable industrial processes. Polysaccharides are already in use, for example, in the food and cosmetic industries, but new application areas are enabled as novel plausible methods for polysaccharide modifications are developed. Desired physico-chemical properties, such as solubility or hydrophobicity, of polysaccharides can often be reached by introducing functional groups to the polysaccharides (Cunha & Gandini, 2010a, 2010b; Xu et al., 2010, 2011).

An interesting approach to chemical modification of polysaccharides is the introduction of highly reactive carbon–carbon double or triple bonds. Both functional groups can be subsequently reacted enabling anchoring of desired functionalities to the polysaccharide, i.e. stimuli-responsive moieties, hydrophobic tails, bioactive, and UV-sensitive compounds. These may find applications in biomedical, food, intelligent packaging, and bio-detection applications (Maharjan et al., 2008; Cheng et al., 2010). The double bonds in allylated starch have been used to form epoxides (Huijbrechts et al., 2010) and for co-polymerization with acrylic monomers (Bhuniya, Rahman Md, Satyanand, Gharia, & Dave, 2003), while the triple bonds of propargylated starch have been further reacted using "click-chemistry" (Elchinger et al., 2011; Tankam, Müller, Mischnick, & Hopf, 2007).

The allylation of carbohydrates was first reported by Tomecko and Adams (1923). In order to introduce reactive groups to polysaccharides, the authors formed allyl ethers of e.g. dextrin, starch, and cellulose by reacting the polysaccharides with allyl bromide in alkaline conditions. Allyl ethers of polysaccharides have also been prepared using allyl glycidyl ether (Ameye et al., 2002; Duanmu, Gamstedt, & Rosling, 2007; Huijbrechts et al., 2007) and in different solvent systems, such as *N,N*-dimethylacetamide and lithium chloride (LiCl/DMAc) (Lin & Huang, 1992) or dimethyl sulfoxide/tetrabutylammonium fluoride trihydrate (Heinze, Lincke, Fenn, & Koschella, 2008).

Aldehydes and ketones can be allylated using a Barbier–Grignard type reaction, where the carbonyl group

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reacts with an allyl halide in the presence of a metal mediator to form a homoallyl alcohol (Pétrier & Luche, 1985; Li, 1996). In carbohydrate chemistry, this reaction has previously been applied on unprotected monosaccharides to extend aldoses, and in synthesis of bioactive compounds (Balla, Zamyatina, Hofinger, & Kosma, 2007; Chan & Li, 1992; Gao, Martichonok, & Whitesides, 1996; Gordon & Whitesides, 1993; Schmid & Whitesides, 1991). We have recently allylated the C-6 of methyl galactopyranoside through combining enzymatic oxidation by galactose oxidase (GO, E.C. 1.1.3.9) with indium mediated allylation (Leppänen et al., 2010). Since both the enzymatic oxidation and the metal mediated allylation reaction are performed in water, it is possible to obtain allylated polysaccharides in a one-pot reaction. In addition, the same indium mediated procedure could also be applied on propargyl bromide to introduce alkynyl groups to the polysaccharide. The enzyme GO catalyses the oxidation of primary alcohols to corresponding aldehydes (Whittaker, 2003). GO is regioselective towards the hydroxyl at C-6 of galactose units, and it can, in combination with catalase and horse radish peroxidase (HPR), be used for selective oxidation of galactopyranosyl units in polysaccharides (Hartmans et al., 2004; Parikka et al., 2010). Polysaccharides that thus can be selectively oxidized by GO are, for example, spruce *O*-acetyl-galactoglucomannan (GGM), guar galactomannan (GM), and (galacto)xyloglucan (XG). Especially the use of GGM is of interest for the Nordic forest industry due to the availability of the polysaccharide in pulping of softwood and in particular, spruce.

We present here a novel method for introduction of allyl groups to polysaccharides. The galactose-containing polysaccharides GGM, GM, and XG were successfully allylated using enzymatic oxidation combined with indium mediated allylation. Reactions with allyl bromide, crotyl chloride, and cinnamyl chloride were assessed for the introduction of different functional groups. In addition, the same procedure was applicable when performing reactions using propargyl bromide. The reaction products were analyzed by a MALDI–MS method, by which the approximate conversion to reaction products could feasibly be observed. This is the first report on the utilization of MALDI–MS in the analysis of enzymatically and chemically modified GGM, and GM derivatives.

2. Materials and methods

2.1. Materials

GGM was prepared from spruce thermomechanical pulp (TMP) by a large laboratory-scale method modified from the method reported by Willför, Rehn, Sundberg, Sundberg, & Holmbom (2003). In short, a suspension of TMP in hot tap water was stirred for 3 h and the pulp was removed. The extract water was purified from colloidal wood resin, and aromatic residues using a cationic coagulant (Raifix 120, Raisio Chemicals Oy, Finland) and XAD-7 resin (Amberlite, Rohm and Haas, UK). The water was concentrated by ultra-filtration before GGM was isolated by precipitation in ethanol and air dried.

XG of reduced molar mass (M_w 1.7×10^4 g/mol, M_w/M_n 2.0) was prepared from tamarind seed xyloglucan (Innovasynth Technologies Ltd., India) by digestion with the xyloglucan-specific endo-glucanase (EGase, EC 3.2.1.151, specific activity 70,680 U/25 g) from *Chrysosporium lucknowense* (xgl1). EGase was purchased from Dyadic NL, Wageningen, NL.

Raffinose and GM were purchased from Sigma–Aldrich, and were used without further purification.

Galactose oxidase (GO) for oxidation of GGM and GM was a gift from Hercules (Barneweld, Netherlands). It was produced by *Pichia pastoris* carrying the gene encoding GO from *Fusarium* spp., and used without further purification. As the activity of GO was not known, the reported specific activity of a similar preparation was used for the estimation of the GO amounts (26 U/mg). For the oxidation of XG, GO from *Fusarium graminearum* was produced recombinantly in *Pichia pastoris* as described previously (Spadiut, Olsson, & Brumer, 2010). Horseradish peroxidase (P8250, Type II, 181 U/mg) and catalase (C30, from bovine liver, 22,000 U/mg) were purchased from Aldrich. Endo-1,4-β-mannanase (1,4-β-D-mannan mannanohydrolase) (*Cellvibrio japonicus*), EC 3.2.1.78 (420 U/mg at 40 °C, pH 7) was purchased from Megazyme.

All chemicals were of commercial grade. Allyl bromide was purchased from Merck (Hohenbrunn, Germany), crotyl chloride, cinnamyl chloride and propargyl bromide (80 wt.% in toluene) from Sigma–Aldrich (Steinheim, Germany). Indium powder (100 mesh) was purchased from Sigma–Aldrich (Steinheim, Germany).

2.2. Experimental methods

2.2.1. Enzymatic oxidation

The polysaccharides were enzymatically oxidized using galactose oxidase in combination with horseradish peroxidase (HRP) and catalase, as previously reported by Parikka and Tenkanen (2009) and Parikka et al. (2010). In short, the polysaccharide was dissolved in water (1–10 mg/mL), and the enzymes GO, catalase, and HRP were added. The amount of GO was related to the approximate amount of terminal galactose present in the polymer (0.052 U of GO/1 mg of galactose). The dosage of catalase was 115 U/mg and HRP 1.5 U/mg. The solutions were stirred at room temperature for 48 h, and were then heated in boiling water for ca. 10 min to inactivate the enzymes. The oxidized polysaccharides were not isolated; the indium mediated allylations were performed directly in the solutions in which the oxidations were done.

2.2.2. General procedure for indium-mediated allylation and propargylation

The amounts of reactants were related to the approximate molar amount of oxidized galactose units in the solution. Plain water was used as solvent in most reactions. Allylation reactions of oxidized raffinose were also done using methanol (MeOH) or tetrahydrofuran (THF) as co-solvents (co-solvent-H_2O 1:4, v/v). Oxidized GGM was also allylated in HCl (0.1 M), or using THF as co-solvent (THF-H_2O 1:4, v/v).

The typical amount of sample was ca 10 mg. To the aqueous solution containing the oxidized polysaccharide (1–10 mg/mL), indium powder (5–10 equiv.) and the allylic or propargylic halide (5–10 equiv.) were added, and the solution was stirred at room temperature or at 55 °C for 24–48 h. The allylated polysaccharide was isolated by precipitation in ethanol (ethanol-water 10:1, v/v), washed twice with ethanol–water (10:1, v/v), and dried by freeze-drying or under N_2.

2.2.3. Indium-mediated allylation of oxidized GGM

To the solution of oxidized GGM in water (~6 mg/mL, 25 mL), indium powder (60 mg) and allyl bromide (60 mg) were added, and the solution was stirred at 55 °C for 24 h. The allylated GGM was then filtered through a glass microfiber filter to remove the indium before it was isolated by precipitation in ethanol (ethanol–water 10:1, v/v), washed twice with ethanol–water (10:1, v/v), and dried by under N_2 and in a vacuum desiccator. The product was obtained in 54% yield as a white powder.

2.3. Analytical methods

2.3.1. The carbohydrate content

The carbohydrate content of the modified polysaccharides was determined by gas chromatography (GC) and by gas