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Oxidation of mannosyl oligosaccharides by hydroxyl radicals as assessed by electrospray mass spectrometry

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ABSTRACT

The hydroxyl radicals are widely implicated in oxidation of carbohydrates during biological and industrial processes being responsible for their structural modifications and causing functional damage. The identification of intermediate oxidation products is hampered by a lack of reliable sensible methods for their detection. In this study, the oxidation of two models of galactomannans (Man_3 and $GalMan_2$) has been studied in reaction with hydroxyl radical generated by Fenton reaction. The oxidation patterns were assessed using preparative ligand-exchange/size-exclusion chromatography (LEX/SEC) coupled with tandem electrospray mass spectrometry (ESI-MS/MS). This allowed the identification of derived oligosaccharides (OS) containing hexuronic, hexonic, pentonic and erythronic acid residues and neutral OS bearing hydroperoxy, hydrated carbonyl moieties and residues from pyranosyl ring cleavage. The depolymerization products have been also detected upon oxidation of oligomers. This study allowed developing a simple, effective 'fingerprinting' protocol for detecting the damage done to mannans by oxidative radicals.

1. Introduction

The hydroxyl radicals ($HO^{\cdot}$) are recognized highly active species in oxidation of carbohydrates with oxygen, ozone or peroxides in many biological[1] and industrial processes.[2] Thus, $HO^{\cdot}$ are suspected to be responsible for the non-enzymatic scission of polysaccharides in biological systems with hyaluronate,[3,4] chitosan,[5] pullulan, carboxymethylcellulose, welan, scleroglucan,[6] xyloglucan, and pectin.[7–9] $HO^{\cdot}$ are accused for the strong depolymerization of polysaccharides during oxygen, ozone or hydrogen peroxide bleaching of chemical pulps for the papermaking[10,11] and promote the thermal degradation of cellulose.[12] These reactive species were also proposed to be involved in oxidation of galactomannans during coffee roasting.[13]

A variety of secondary end products may be formed from carbohydrate-derived free radical afforded after C–H bond scission with $HO^{\cdot}$. Major studies on the oxidized intermediates of carbohydrates with $OH^{\cdot}$ have been carried using monomeric sugars and much less with oligo- and polysaccharides.[14,15] The majority of detected oxidation products are low molecular weight compounds generated from depolymerization and degradation of carbohydrates.[7,14,15] Some of these products, such as uloses, aldonic and uronic acids, tetrose and tetrulose derived from parent hexoses and pentoses have been identified.[14–19] The structural changes in the carbohydrates can modulate significantly their reaction patterns, as for example hexuloses are more reactive than the original reducing end units in redox and S_N reactions[20,21] and hexonic acids are not reactive at all in those reactions.[22] Scarce structural details exist for the oligo- and polysaccharides that have been subjected to the radical reactions (oxidation intermediates) and did not degrade to monomeric units.[7–9,14,16] Major difficulties in analysing those intermediates, which bring precious information about reaction pathways are their relatively low abundance and stability during isolation/derivatization.

Mass spectrometry (MS) has been widely considered during the last decade for the structural characterization of oligosaccharides.[23–25] Electrospray mass spectrometry (ESI-MS) and tandem mass spectrometry ($ESI\text{-}MS^n$) have been recognized particularly advantageous tool for the structural analysis of low abundance (up to picomole levels) oligomers possessing an increased molecular mass without their previous derivatization and(or) partial depolymerization.[24,25]

The main goal of this work was to study the oxidation patterns of two isomeric trisaccharides, Man_3 and $GalMan_2$ (Scheme 1) induced by the hydroxyl radical generated under conditions of

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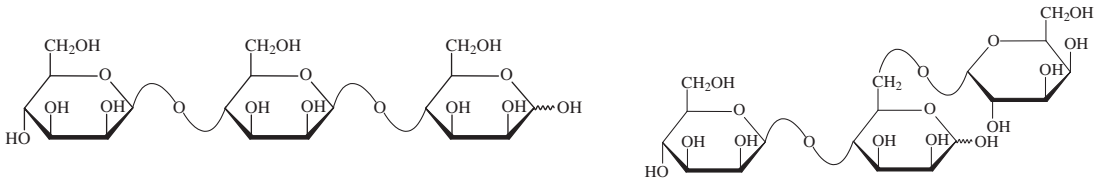

Man_3 $GalMan_2$

Scheme 1. Isomeric mannosyl containing trisaccharides, Man_3 and $GlalMan_2$ that were induced to oxidation by the hydroxyl radical generated under Fenton conditions (H_2O_2/Fe^{2+}).

Fenton reaction. Oxidation products were separated using semi-preparative ligand-exchange/size exclusion chromatography (LEX/SEC) and analyzed by electrospray mass spectrometry (ESI-MS) and tandem mass spectrometry (MS/MS). The results obtained allowed developing a simple, effective 'fingerprinting' protocol for detecting the damage done to galactomannans by HO· radicals, using the model oligosaccharides Man_3 and $GalMan_2$.

2. Materials and methods

2.1. Reagents

1,4-β-D-Mannotriose (Man_3) and 6^1-α-D-galactosyl-mannobiose ($GalMan_2$) were supplied from Megazyme Comp. (Wicklow, Ireland). Ferrous chloride and hydrogen peroxide (30%) were obtained from Merck Comp. (Darmstadt, Germany). Xylenol orange (XO) (Fluka Chem. Comp.), $FeSO_4{\cdot}7H_2O$ (Merck (Darmstadt, Germany)) and H_2SO_4 were used for Fox method. Triphenylphosphine was used for reduction of the hydroperoxydes. All solvents used were HPLC grade, and the solutions used were prepared with MilliQ high purity water.

2.2. Oxidation reaction

In this work, the oxidation of two different trisaccharides Man_3 and Man_2Gal (Figs. 1 and 2) was induced by the hydroxyl radical generated under Fenton Reaction conditions. Briefly, the trisaccharides (2 mM) were incubated at 37 °C in 200 μL of aqueous medium containing $FeCl_2$ (40 μM) and H_2O_2 (50 mM). The reaction mixtures were left to react overnight. The hydroperoxide derivatives obtained after the oxidation reaction were degraded while incubating the solution of oxidized trisaccharides (ca. 50 μg/mL) for 20 min at 4 °C with solution of triphenylphosphine (TPP) (1 mg/mL in ethanol).

2.3. Quantification of the hydroperoxides

An aliquot of trisaccharides solution (50 μL) before and after reduction with TPP were incubated with Fox reagent (950 μL) for 30 min in dark. The Fox reagent was prepared with 100 μM XO, ferrous sulfate (250 μM; 6.95 mg), 25 mM of sulfuric acid (139 μL), and 100 μM of sorbitol in aqueous solution (total volume 100 mL). The absorbance at λ = 560 nm was assessed using a Thermo Fisher Scientific GENESYS 10 Bio UV–vis Spectrophotometer.

2.4. Chromatographic separation

The products from oxidation of oligosaccharides were separated by semi-preparative LEX/SEC (pump Knauer K-1001) using a Shodex sugar KS 2002 (Showa Denko K.K.) column (300 mm × 20 mm) at 30 °C and ultra-pure water (pH 5.8 adjusted with H_2SO_4 diluted solution) as eluent. A flow rate of 2.80 mL/min and a detector RI (Knauer K-2401) were used. The injected sample volume was 500 μL. Approximately 1 mg of oxidation products was injected per run. Fractions obtained were analyzed by ESI-MS and ESI-MS/MS.[26,27]

2.5. Electrospray ionization mass spectrometry (ESI-MS and MS/MS)

The ESI-MS and ESI-MS/MS were carried out on a Q-TOF2 hybrid tandem mass spectrometer (Micromass, Manchester, UK). Samples were introduced at a flow rate of 10 μL/min into the electrospray source. In MS and MS/MS experiments TOF resolution was set to

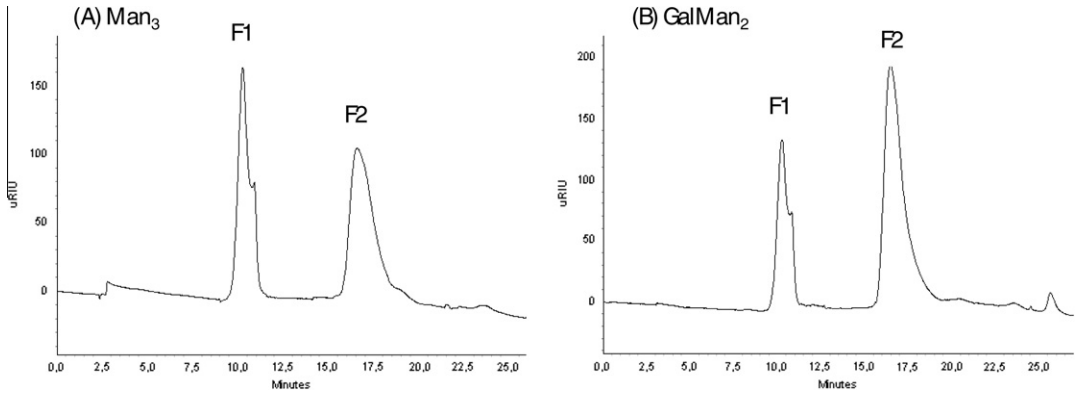

Figure 1. LEX/SEC chromatograms of oxidized Man_3 (A) and $GalMan_2$ (B).