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Introducing porous graphitized carbon liquid chromatography with evaporative light scattering and mass spectrometry detection into cell wall oligosaccharide analysis

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ABSTRACT

Separation and characterization of complex mixtures of oligosaccharides is quite difficult and, depending on elution conditions, structural information is often lost. Therefore, the use of a porous-graphitized-carbon (PGC)-HPLC-ELSD-MS^n-method as analytical tool for the analysis of oligosaccharides derived from plant cell wall polysaccharides has been investigated. It is demonstrated that PGC-HPLC can be widely used for neutral and acidic oligosaccharides derived from cell wall polysaccharides. Furthermore, it is a non-modifying technique that enables the characterization of cell wall oligosaccharides carrying, e.g. acetyl groups and methylesters. Neutral oligosaccharides are separated based on their size as well as on their type of linkage and resulting 3D-structure. Series of the planar β-(1,4)-xylo- and β-(1,4)-gluco-oligosaccharides are retained much more by the PGC material than the series of β-(1,4)-galacto-, β-(1,4)-manno- and α-(1,4)-gluco-oligosaccharides. Charged oligomers such as α-(1,4)-galacturonic acid oligosaccharides are strongly retained and are eluted only after addition of trifluoroacetic acid depending on their net charge. Online-MS-coupling using a 1:1 splitter enables quantitative detection of ELSD as well as simple identification of many oligosaccharides, even when separation of oligosaccharides within a complex mixture is not complete. Consequently, PGC-HPLC-separation in combination with MS-detection gives a powerful tool to identify a wide range of neutral and acidic oligosaccharides derived from various cell wall polysaccharides.

1. Introduction

Plant cell wall polysaccharides play an important role in the structure and functionality of the whole plant [1,2]. The elucidation of their biosynthesis as well as a better understanding of their functional properties and enzymatic degradability is essential for the use of these polysaccharides by the food and non-food industry. Therefore, detailed analysis of their precise structure is essential [3].

Since monomeric sugar composition analysis alone cannot deliver any structural information of the parental polysaccharide, the analysis of these polysaccharides is mostly done after partial degradation by chemical or enzymatic treatment into oligosaccharides. Nowadays, high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) is frequently used for oligosaccharide analysis [4,5]. However, the high pH eluent used for separation and detection will result in elimination of methylesters and acetyl groups of the oligomers under investigation. In addition, HPAEC-PAD is not fully MS-compatible due to the high salt content used within the eluents. As methyl- and acetyl-substitutions are important for the function and the properties of polysaccharides, it is necessary to have analytical methods that leave the structural features intact [6].

Recently, oligosaccharide analysis using capillary electrophoresis (CE) coupled to laser induced fluorescence (LIF) and mass spectrometry (MS) detection has been described [7–9]. This technique is very powerful and provides good separation of many oligosaccharides derived from plant cell wall polysaccharides in a short time but may not be commonly available. In addition, sample preparation includes time-consuming labeling of the oligosaccharides with a fluorescent chromophore by which non-reducing sugars are excluded from analysis. Furthermore, acidic oligosaccharides derived from pectin are being eluted rather rapidly due to the additional charges and an adequate separation is rather difficult to obtain [9].

Another available technique for the separation and identification of neutral oligosaccharides is the gradient elution using RP-HPLC coupled to an evaporative light scattering (ELS) detector and a mass spectrometer (MS) as described by Kabel et al. [10]. However, due to the high polarity no retention could be achieved for acidic oligosaccharides derived from pectins. Recently, Hem-

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ström and Irgum [11] reviewed the use of hydrophilic interaction chromatography (HILIC) for the separation of certain classes of oligosaccharides. Although the use of HILIC within oligosaccharide analysis seems to be promising, solubility of higher oligomers in the starting eluent (e.g. 80% organic modifier) might be a problem.

Porous graphitized carbon (PGC)-HPLC columns have been shown to be able to separate maltodextrins, fructo-oligosaccharides, human milk oligosaccharides, oligosaccharides derived from glycoproteins and even very polar sugar phosphates and glucosinolates [12,13]. The ability of PGC material in retaining very polar components is based on the polar retention effect of graphite, which enables electron transfer between the carbon material and the acidic analytes as described by Pereira [14].

As analysis of neutral and acidic oligosaccharides still lacks a rapid, versatile and widely available technique, this research aimed at the separation and identification of a broad range of neutral and acidic oligosaccharides within complex mixtures derived from plant cell wall polysaccharides. To this end, the use of PGC-HPLC in combination with ELS- and MS-detection was explored.

2. Experimental

2.1. Materials

2.1.1. Neutral and acidic homoglycan oligosaccharides

The following components have been used as such: maltodextrins (α-1,4-gluco-oligomers) with an average degree of polymerization (DP) of 5 (AVEBE, Veendam, The Netherlands) and cellulodextrins (β-1,4-gluco-oligomers) with DP 2–7 (a kind gift of Dr. Vladimir Farkas, Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovakia).

α-1,5-Arabino-oligomers (DP 1–8) were produced by partial enzymatic degradation of linear arabinan with α-1,5-arabinanase (*Aspergillus aculeatus*) [15]. A mixture of acetylated β-1,4-xylo-oligomers derived from *Eucalyptus* wood has been produced as described by Kabel et al. [16] (fraction Euc NI A). β-1,4-Manno-oligomers were produced by partial enzymatic degradation of β-1,4-mannan derived from Ivory Nut (Megazyme, Bray, Ireland) with β-1,4-mannananase (*Aspergillus niger* [17]). β-1,4-Galacto-oligomers were produced by partial enzymatic degradation of pectic potato galactan (Megazyme, Ireland) with β-1,4-galactanase (*A. niger* [18]).

Purified fractions of α-1,4-galacturonic acids (DP 2–10) as described by Van Alebeek et al. [19] have been used.

2.1.2. Neutral and acidic heteroglycan oligosaccharides

Xyloglucan oligosaccharides derived from tamarind were obtained after digestion with xyloglucan specific endo-glucanase (XEG, *A. aculeatus*) as described by Hilz et al. [8].

Partially esterified α-(1,4)-galacturonic acid oligosaccharides were obtained by digestion of low methylesterified pectin (DM 30) with α-(1,4)-endo-polygalacturonase (*Kluyveromyces fragilis marxianus* [20]).

Oligosaccharides derived from rhamnogalacturonan I were obtained by saponification of pectic modified hairy regions (MHR-B; [21]) with 0.1 M sodium hydroxide followed by treatment with rhamnogalacturonan hydrolase in overdose [22].

Xylogalacturonan oligomers were produced by treating xylo-galacturonan from *gum tragacanth* [23] with an overdose of xylogalacturonan hydrolase (XGH [24]).

2.2. HPLC-ELSD-MS^n

Liquid chromatography was performed on a Thermo Accela UHPLC system (Waltham, MA, USA) equipped with a Hypercarb column (PGC, 100 mm × 2.1 mm; 3 μm, Thermo Electron Corporation, San José, USA) in combination with a Hypercarb guard column (10 mm × 2.1 mm; 3 μm, Thermo Electron Corporation). Elution was performed with a flow of 0.4 mL/min and a column oven temperature of 70 °C. The injection volume was set to 10 μL. The following eluents were used: Millipore water (A), acetonitrile (B) and 0.2% (v/v) trifluoroacetic acid in water (TFA, C), to all eluents 25 μM sodium acetate was added to ensure sodium adducts of all components. The following gradient was used: 0–1 min, isocratic 100% A, 1–15 min, linear from 0 to 27.5% (v/v) B, 15–28 min linear from 27.5 to 60% B and concomitant linear from 0 to 10% (v/v) C, 28–31 min linear from 60 to 80% B and from 10 to 20% C, 31–35 min isocratic 80% B and 20% C, 35–36 min from 80% B and 20% C to 100% A, 36–41 min equilibration with 100% A.

The PGC-column was coupled to a 1:1-splitter (Accurate, Dionex, Sunnivale, USA) directing the eluent both to the evaporative light scattering detector (ELSD 85, Sedere, Alfortville Cedex, France) and to the ESI-MS^n-detector (LTQ XL MS, ion trap, Thermo Scientific, San Jose, CA, USA). The ELSD micro flow nebulizer (Sedere, Alfortville Cedez, France) had a gas pressure of 3.5 bar and a gas flow of 1.75 L/min. The drift tube temperature of the ELSD was set to 50 °C and the gain to 12. MS-detection was performed in positive mode using a spray voltage of 4.5 kV and a capillary temperature of 260 °C and auto-tuned on malto-pentaose (m/z 851). The MS^n-experiments were performed based on dependent scan-settings (Xcalibur software, Thermo Electron Corporation, San José, USA). The addition of sodium acetate (25 μM) in combination with the use of TFA introduces a significant amount of noise due to the polymerization of sodium trifluoroacetate ($\Delta m/z$ 136). The m/z-values originating from the sodium-TFA-complexes are excluded from the online-MS^2 measurements by means of Xcalibur software (Thermo Electron Corporation, USA). The column was regenerated with 70 column volumes of tetrahydrofuran after each series of analysis [25].

3. Results and discussion

Various mixtures of oligosaccharides have been analyzed with a PGC-HPLC column in order to investigate the potential of porous graphitized carbon material in the analysis of oligosaccharides derived from a broad range of neutral and acidic oligosaccharides and to understand the mechanism involved in this separation.

3.1. Optimization of elution conditions for separation of various cell wall derived oligosaccharides

Different conditions including various compositions of organic and acidic modifiers as well as different temperatures have been investigated for their ability to elute and separate the broad range of cell wall derived neutral and acidic oligosaccharides.

Water–acetonitrile (ACN)-gradients have been described before to be effective to separate oligosaccharides of various sources [13,26]. During this research the steepness of the water–ACN-gradient has been varied and an increase of 2% ACN/min has been selected as a suitable compromise for the separation of all neutral oligosaccharides under investigation in reasonable run times. In Fig. 1A–C typical chromatograms of α-1,4-linked maltodextrins (Fig. 1A), β-1,4-linked cellulodextrins (Fig. 1B) and α-1,5-linked arabinan oligomers (Fig. 1C) are presented.

α-(1,4)-Galacturonic acid oligomers are strongly retained by the PGC material and an acidic modifier is needed for elution. During this research three different acidic modifiers have been investigated, namely acetic acid, formic acid and TFA. The use of acetic acid did not result in an elution of all charged oligomers under investigation, whereas formic acid and TFA enable the elution of