Carbohydrate Polymers 94 (2013) 791–799

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

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

Profiling of soluble neutral oligosaccharides from treated biomass using solid phase extraction and LC–TOF MS

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ARTICLE INFO

Article history:
Received 16 October 2012
Received in revised form 1 February 2013
Accepted 5 February 2013
Available online 13 February 2013

Keywords:
Ammonia fiber expansion (AFEX) pretreatment
Arabinoxylans
Corn stover
Glucans
Porous graphitized carbon (PGC)
Solid phase extraction (SPE)

ABSTRACT

Thermochemical pretreatments of cellulosic biomass are known to improve cell wall enzymatic digestibility, while simultaneously releasing substantial amounts of soluble oligosaccharides. Profiling of oligosaccharides released during pretreatment yields information essential for choosing glycosyl hydrolases necessary for cost-effective conversion of cellulosic biomass to desired biofuel/biochemical end-products. In this report we present a methodology for profiling of soluble neutral oligosaccharides released from ammonia fiber expansion (AFEX™)-pretreated corn stover. Our methodology employs solid phase extraction (SPE) enrichment of oligosaccharides using porous graphitized carbon (PGC), followed by high performance liquid chromatography (HPLC) separation using a polymeric amine based column and electrospray ionization time-of-flight mass spectrometry (ESI-TOF-MS). For structural elucidation on the chromatographic time scale, nonselective multiplexed collision-induced dissociation was performed for quasi-simultaneous acquisition of oligosaccharide molecular and fragment masses in a single analysis. These analyses revealed glucans up to degree of polymerization (DP) 22 without modifications. Additionally, arabinoxylans up to DP = 6 were detected in pretreated biomass extracts (post-enzymatic digestion). Cross-ring fragment ion abundances were consistent with assignment of linkages between sugar units in glucans and also xylose backbone in arabinoxylans as 1–4 linkages. Comprehensive profiling of soluble oligosaccharides also demonstrated decreases in levels of acetate esters of arabinoxylan oligosaccharides with concomitant increases in nonacetylated oligosaccharides that were consistent with earlier observations of 85% release of acetate esters by AFEX™ pretreatment.

1. Introduction

The biosynthesis of carbohydrate polymers represents one of the most prolific biochemical transformations on Earth. It has been estimated that natural plant biosynthesis generates more than 10^{11} tons of biomass per year (Duchesne & Larson, 1989; Pauly & Keegstra, 2008) with around half of this consisting of carbohydrate polymers cellulose and hemicelluloses. Carbohydrate polymers are responsible for plant cell wall structure and strength, storage of biochemical energy in the form of starch, and production of materials with far-reaching application including gelling and emulsifying agents and as drug delivery agents (Sinha & Kumria, 2001). Humankind exploits only a small fraction of this biomass, and as a result, cell wall polysaccharides are attractive renewable resources. Abundance alone makes cell wall polysaccharides attractive renewable feedstocks for bioenergy, and specialty products.

Plant cell wall oligosaccharides are materials of daunting complexity, being composed of various (5 or 6 carbon) sugar monomers with different degrees and positions of branching, assorted chemical modifications including acetylation and feruloylation (Ishii, 1997), and heterogeneity in molecular mass. These

Abbreviations: AFEX, ammonia fiber expansion; AFEXTCS, AFEX-treated corn stover; AP1, aperture 1; AX, arabinoxylan; CID, collision-induced dissociation; DP, degree of polymerization; ESI, electrospray ionization; HILIC, hydrophilic interaction chromatography; HPAEC, high performance anion-exchange chromatography; IM, ion mobility; MALDI, matrix-assisted laser desorption ionization; PGC, porous graphitized carbon; SPE, solid phase extraction; TOF, time-of-flight; UTCS, untreated corn stover.

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http://dx.doi.org/10.1016/j.carbpol.2013.02.005

factors have strong influence on their solubility and digestibility (Scheller & Ulvskov, 2010). Conversion of complex polysaccharides, particularly those from cellulosic biomass, to fermentable monosaccharides is often inefficient owing to chemical modifications including formation of diferulate crosslinks that take place in vivo during cell wall assembly. Yields of fermentable products are improved following application of hydrolytic and ammonolytic pretreatments including acid, alkali, and AFEX™, which remove acetyl and phenolic acid esters from modified polysaccharides. AFEX involves treating biomass with liquid ammonia in a pressurized reactor at temperatures of 100 °C and above. The process causes physical changes in cellulose crystallinity and partial depolymerization of hemicelluloses. Recycling of ammonia leaves behind a glycopolymer-rich material with reduced recalcitrance to hydrolysis (Sousa, Chundawat, Balan, & Dale, 2009). In order to better predict yields of conversion of cell wall biomass to fermentable sugars and to optimize design of efficient biorefineries, comprehensive profiling in combination with yield determination of mono- and poly-saccharides generated by pretreatment and during enzymatic processing is needed that goes beyond destructive conversion of glycopolymers to monomeric sugars.

Profiling of oligosaccharides derived from processing of cell walls starts best by defining the molecular masses of the individual components in a mixture of oligosaccharides. Modern mass spectrometry readily provides molecular mass information through application of soft ionization methods including matrix-assisted laser desorption ionization (MALDI) (Harvey, 1999) or electrospray ionization (ESI) (Reinhold, Reinhold, & Costello, 1995). When soft ionization is combined with CID to generate fragment ions, the resulting information allows for characterization of sequences of sugar monomers, linkages between sugars based on cross-ring fragment masses, and presence of branching. This approach has been exploited to investigate structures of oligosaccharides derived from plant tissues (Fernandez, Obel, Scheller, & Roepstorff, 2003, Fernandez, Obel, Scheller, & Roepstorff, 2004; Harrison et al., 2011; Maslen, Goubet, Adam, Dupree, & Stephens, 2007; Van Dongen, Van Eylen, & Kabel, 2011). Most of these reports describe characterization of products of enzymatic digestions using soft ionization and MS/MS, frequently employing permethylation to improve information content in the mass spectra. One report demonstrated detection of fructans with DP > 100 using HPLC based on PGC and electrospray ionization (Harrison et al., 2011). More recently, the combination of mass spectrometry with ion mobility (IM) separations of ions allowed for discrimination of oligosaccharides by shape, and not just mass (Munisamy, Chambliss, & Becker, 2012). Despite great advances in IM technology, this approach has yet to achieve resolution of the vast array of isomeric oligosaccharides without prior physical separation.

Another fast and powerful tool for structural identification/confirmation of analytes employs quasi-simultaneous acquisition of exact masses at high and low collision energies in a single analysis without mass filtering (MS^E) (Plumb et al., 2006). Fast data acquisition provided by time-of-flight mass spectrometry allowed for extension of this technique to use more than two collision conditions, termed multiplexed collision-induced dissociation. This approach yields CID mass spectra using multiple collision energies on the chromatographic time scale, and has driven discoveries of new plant metabolites and genes responsible for metabolite accumulation (Gu, Jones, & Last, 2010; Schilmiller et al., 2010). To our knowledge, multiplexed CID has not been reported for oligosaccharides.

Separation of oligosaccharides before mass spectrometry is essential for comprehensive oligosaccharide profiling owing to the complexity of plant oligosaccharide mixtures. Some notable successes in separation of oligosaccharides were achieved through use of hydrophilic interaction chromatography (HILIC) (Churms, 1996; Karlsson, Swerup, & Sandberg, 2008.; Leijdekkers, Sanders, Schols, & Gruppen, 2011). High-pH anion exchange chromatography (HPAEC) has emerged as another common carbohydrate separation method that takes advantage of partial ionization of oligosaccharides at elevated pH (Cataldi, Campa, & De Benedetto, 2000; Guignard et al., 2005; Lee, 1996; van der Hoeven et al., 1998), but the common nonvolatile mobile phase additives that achieve high pH are incompatible with electrospray ionization unless they are removed post-column. PGC has proved to be a suitable chromatographic stationary phase for retention of very polar compounds owing to both hydrophobic and electronic-type interactions between the analyte and the PGC surface (Hennion, 2000; West, Elfakir, & Lafosse, 2010). PGC columns have been used to enrich or separate various sugars and sugar polymers including sugar phosphates from *Arabidopsis thaliana* (Antonio et al., 2007), cell wall oligosaccharides (Westphal, Schols, Voragen, & Gruppen, 2010) and human milk oligosaccharides (Strum, Aldredge, Barile, & Lebrilla, 2012; Zhang, Xie, Hedrick, & Lebrilla, 2004). PGC separations offer the advantage that mobile phases are often compatible with mass spectrometry analyses.

About a decade ago, the introduction of the Prevail Carbohydrate ES column (Alltech), a polymeric column with amine groups, yielded separations that resolved a variety of neutral mono- and oligosaccharides. This column yields separations similar to normal phase chromatography using water and acetonitrile as solvents, and these are compatible with ESI mass spectrometry. Most of the applications of this column reported to date focused largely on analyses of mono- and disaccharides (Agblevor, Murden, & Hames, 2004; Kalay et al., 2012; Slimestad & Vagen, 2006; Vinjamoori, Byrum, Hayes, & Das, 2004; Wan & Yu, 2007).

As mentioned above, pretreatment of biomass improves yields of conversion of carbohydrates to fermentable sugars, but the fundamental relationships between the severity of pretreatment and the digestibility of products remains uncertain. Pretreatment processes release complex mixtures of substances including phenolics, Maillard reaction products, and mono- and oligosaccharides (Chundawat et al., 2010). Initial efforts that profiled soluble carbohydrates using a Bio-Rad Aminex 42-A column resolved carbohydrates with DP < 5, but larger oligomers were not resolved. Furthermore, chromatographic peak areas of oligosaccharides detected using refractive index and mass spectrometric detection did not account for the total sugar monomers yielded by acid hydrolysis. This finding led us to suspect that losses of oligosaccharides were occurring during sample processing, and a strategy for oligosaccharide enrichment was pursued using SPE based on PGC as a prelude to LC/MS profiling.

In the current study, soluble neutral oligosaccharides including xylans and glucans were profiled in extracts of AFEX™-pretreated corn stover (AFEXTCS). Enrichment of larger oligomers using PGC–solid phase extraction (PGC–SPE) followed by analytical separation using a Prevail Carbohydrate ES column coupled to multiplexed collision-induced dissociation mass spectrometry provided a fast technique that yielded rich information for comprehensive profiling of neutral soluble oligosaccharides in extracts of untreated and pretreated plant material.

2. Results and discussion

2.1. Optimization of conditions for PGC–SPE and enrichment of oligosaccharides

Initial LC/MS profiling of carbohydrates released from corn stover during AFEX™ processing detected low mass (DP < 6) oligosaccharides, but mass balance calculations suggested that the analytical result failed to account for more than 75% of glucans.