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Structural characterization of underivatized arabino-xylo-oligosaccharides by negative-ion electrospray mass spectrometry

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Abstract—Various arabino-xylo-oligosaccharides with known substitution patterns were assessed by negative ESI-Q-TOFMS and ESI-ITMS. The CID spectra of linear xylo-oligosaccharides and of nine isomeric mono- and disubstituted arabino-xylo-oligosaccharides established that structures differing in their substitution pattern can be differentiated by this approach. The negative-ion fragmentation spectra of the deprotonated quasi-molecular ions are mainly characterized by glycosidic cleavage ions from the C-series, which provide sequence informations, and by cross-ring cleavage $^{0,2}A_i$ ions, which provide partial linkage information. When the collision energy increased, the cross-ring cleavage $^{0,2}A_i$ ions underwent consecutive loss of water to produce $^{0,2}A_i - 18$ fragment ions and glycosidic cleavage ions of the B-series are also produced besides the C_i ions. Contrary to linear xylo-oligosaccharides, C_i ions, which originate from C-3 monosubstituted xylosyl residues never produce the related cross-ring cleavage $^{0,2}A_i$ ions. Disubstitution at O-2 and O-3 of xylosyl residues appears to enhance the production of the $^{0,2}A_i$ ions compared to monosubstitution. For the differentiation of the mono- and disubstitution patterns of the penultimate xylosyl residue, the relative abundance of the glycosidic cleavage ions at m/z 263 and 299 found on Q-TOF CID spectra plays a relevant role and appears to be more informative than MS^n spectra obtained on a ion trap instrument.

Keywords: Oligosaccharides; Structural isomers; Xylanase; ESI-Q-TOFMS; ESI-ITMS

1. Introduction

Arabinoxylans (AX) are the main non-starch polysaccharides from wheat grain cell walls. They consist of a linear backbone of β-(1→4)-linked D-xylopyranosyl residues. Xylosyl units can be unsubstituted, monosubstituted at C-3 or disubstituted at C-2 and C-3 with α-L-arabinofuranosyl units.[1] In wheat endosperm, AX are partly water-extractable (25%, WE-AX) and exhibit an average arabinose to xylose ratio (A/X) of 0.6. Natural variations in the structure of WE-AX (A/X) ratio have been described. The structure of water unextractable AX (WU-AX), which represent the major part of AX in the cell walls of endosperms is essentially the same but a higher A/X ratio has been reported.[2,3] Variation in the degree of branching and in the spatial distribution of arabinosyl substituents along the xylan backbone is responsible for the biological and physicochemical properties.[1] The structure of arabinoxylans has been investigated in the past decade using ^1H and ^{13}C NMR spectroscopy, and numerous studies have been reported on the structural characterization of oligosaccharides obtained after xylanase digestion of AX.[1,4–11] Although, NMR sensitivity has considerably improved with the introduction of nanoprobe equipments and very high field magnets, this approach still requires large amounts of high purity samples.[12,13]

As an alternative to NMR spectroscopy, FABMS and ESIMS have been used for oligosaccharide characterization. The main advantages of ESIMS are its sensitivity, and capability to assign linkage,[14–18] sequence and

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branching[19,20] or substituent pattern[21–26] informations upon collision-induced dissociation (CID) of underivatized oligosaccharides. Moreover, derivatization, for example, permethylation[27–29] or reducing-end derivatization[30–34] in combination with CID[35–39] proved to be very useful to clearly identify complex branching patterns of oligosaccharides. Recently, the characterization of a complex oligosaccharides mixture obtained by enzymatic digestion of AX from wheat seeds was carried out using MALDI-TOFMS and ESI-Q-TOFMS or ESI-ITMS.[40] Underivatized linear and branched structures could not be distinguished upon CID using positive-ion mode in either Q-TOF or IT instruments. Permethylation of the oligosaccharides followed by ESI-ITMS with multiple MS steps (MS^n) allowed to partially characterize complex xylo-oligosaccharide structures differing by the number of arabinose residues and their spatial arrangement along the xylose backbone. However, additional techniques of derivatization and GC–MS were necessary to assign the position of the Ara*f* substituent at C-3 of the Xyl*p* residue.[41] Some authors have reported that sequence and branching patterns of underivatized oligosaccharides can however be obtained using negative CID-ESIMS/MS.[18,42–45] The main advantage of negative CID MS/MS is to produce a series of C-type glycosidic cleavage ions, which produce in turn a series of related A-type cross-ring cleavage ions carrying linkage information.[14,23,44–46] In addition, specific D-ions arising from double D-type cleavage at the 3-linked glycosidic bond, may also be produced.[42,43] Recently, negative $CIDMS^n$ was successfully applied to the assignment of acetyl groups to O-2 and/or O-3 positions of pectic oligogalacturonides[24] and of the feruloyl group to O-2 or O-5 of arabinosyl residues and to O-6 of a galactosyl-containing disaccharide.[18]

ESI-ITMS and ESI-Q-TOFMS using negative-ion mode are now assessed for the structural differentiation of eight underivatized mono- and disubstituted xylo-oligosaccharides obtained from enzymatic digestion of wheat WE-AX by an endoxylanase from *Trichoderma viride*.[47] Linear, mono- and disubstituted xylo-oligosaccharides were structurally differentiated upon CID using a Q-TOF instrument in MS/MS experiments.

2. Results and discussion

The eight mono- and disubstituted xylo-oligosaccharides released by enzymatic degradation of wheat WE-AX by an endoxylanase from *T. viride*, were purified by size-exclusion chromatography on Biogel P2 alone or combined with semi-preparative HPAEC.[47] They generally contained less than 3–5% of visible contaminants as determined by HPAEC and ESI-Q-TOFMS. Due to the specificity of the action of the enzyme used, the oligosaccharides were obtained with identical sequences at both ends, namely one and two free xylosyl residues at the non-reducing and reducing end, respectively[47] (Chart 1). In addition monosubstitution is only found at O-3 in wheat AX. Consequently, $A1_2X3$, $A1_3X4$, $A2_{3d}X4$ and $A2_{4,3}X5$ are single isomers for DP 4, DP 5, DP 6 and DP 7, respectively. $A2_{4,3}X5$ (DP 7) and $A2_{5,3}X6$ (DP 8), which co-eluted in the conditions of analytical HPAEC, were separated in conditions used for the semi-preparative HPAEC.[47] Anyway, in this specific case, it was easy to select the appropriate ion, on a mass basis, for tandem mass spectrometry as the relevant m/z for these two oligosaccharides were 941 and 1073, respectively. Fortunately, the three isomers of DP 8 were well separated by HPAEC (retention times 21.0; 23.3 and 24.3 min for $A2_{5,3}X6$, $A3_{4,3d}X5$ and $A3_{4d,3}X5$, respectively).

To establish the main fragmentation patterns of oligosaccharides through CID experiment, ^{18}O-labelling was carried out at the reducing end. This allowed to discriminate isobaric ions, especially B_i from Z_j ions and C_i from Y_j ions, according to the nomenclature of Domon and Costello.[48] Only Z_j and Y_j ions, which contain the reducing end, are ^{18}O-labelled and are characterized by a mass increment of 2 Da. Prior analysis performed by negative ESI-ITMS with multiple MS steps revealed to be more time consuming, less sensitive and less informative than ESI-Q-TOFMS regarding the arabinosyl residues distribution along the xylopyranosyl backbone, particularly for oligomers of DP 6 and more. Therefore, most of the results presented in the following section were obtained using ESI-Q-TOFMS. But generally, the main diagnostic fragment ions were also produced using $ESI\text{-}ITMS^n$.

2.1. General features of negative ESI-Q-TOF tandem mass spectrometry of AX and linear xylo-oligosaccharides

The complete MS spectra of linear xylo-oligosaccharides and of AX were generally dominated by the singly charged $[M^*-H]^-$ and $[M^*-H+36]^-$ quasi-molecular ions. The +36 additional mass was attributed to the solvatation of the molecule by two molecules of water. Indeed, MS/MS experiment performed on the $[M^*-H+36]^-$ quasi-molecular ion showed the $[M^*-H]^-$ species as the main daughter ion besides the same glycosidic cleavage ions as produced upon direct fragmentation of the $[M^*-H]^-$ quasi-molecular ion. However, the collision energy necessary to obtain roughly similar MS^2 spectra was in all cases much higher with the dihydrated molecule. In order to obtain a maximum amount of the precursor ions, the first-order spectra were obtained using low collision energy (10 eV). Under these conditions, some fragment ions such as the ion at m/z 299, which may be considered as diagnostic ions in the MS^2 spectrum, were already present on the full MS spectrum.

The collision energy needed to fragment compounds under CID conditions increases as a function of the