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Probing the structural details of xylan degradation by real-time NMR spectroscopy

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ABSTRACT

The biodegradation of abundantly available cell wall polysaccharides has recently received much attention, not least because cell wall polysaccharides are substrates for the human gut microbiota and for environmentally sustainable processes of biomass conversion to value-added compounds. A major fraction of cereal cell wall polysaccharides consists of arabinoxylans. Arabinoxylan and its degradation products are therefore present in a variety of agro-industrial residues and products. Here, we undertook to track the structural details of wheat arabinoxylan degradation with high resolution NMR spectroscopy. More than 15 carbohydrate residues were distinguished in the substrate and more than 20 residues in partially degraded samples without any sample cleanup. The resolution of a plethora of structural motifs *in situ* permits the readout of persisting structures in degradation processes and in products. Reaction progress was visualized for the biodegradation of arabinoxylan by different crude microbial enzyme preparations. The direct observation of structural details in complex mixtures containing arabinoxylan fragments is significant, as such structural details reportedly modulate the health-promoting functions of arabinoxylan fragments.

1. Introduction

The cell wall supports the plant body plan by forming a fiber-glass-like composite of various macromolecules, specifically cellulose, hemicelluloses and pectin polysaccharides alongside lignin, a polymer of aromatic alcohols (Cosgrove, 2000). The biological function of plant cell wall polysaccharides is the construction of a strong and flexible structure, the formation of a barrier to pathogens and the storage of metabolic energy that can be remobilized in some tissues, for instance in the cereal grains (Burton, Gidley, & Fincher, 2010; Cosgrove, 2000; Scheller & Ulvskov, 2010). Recently, plant cell wall degradation has received much additional attention due to the role of cell walls as the main source of dietary fiber and as renewable biomass sources (Burton et al., 2010). Due to their wide abundance, polysaccharides of plant cell walls are of economic interest as the starting materials for various products and processes.

Unlike cellulose, hemicelluloses and pectin form irregular branched structures with a limited tendency to aggregate (Burton et al., 2010). Due to this irregularity, the structural analysis of hemicellulose (xylan being the main hemicelluose component) and pectin structure is both challenging and labor-intensive. This analysis usually proceeds *via* chemical or enzymatic fragmentation, subsequent purification of fragments and deduction of chemical structure in the purified fragments (Bauer, Vasu, Persson, Mort, & Somerville, 2006). In this work-stream, the preparative fractionation of oligosaccharides has remained a limiting step.

Beyond the structural analysis of polymers, detailed insights into polysaccharide degradation pathways are of additional relevance as they help in the controlled production of oligosaccharides with desired qualities. As an example, cereal-derived arabinoxylan oligosaccharides act as prebiotics by supporting the growth of bifidobacteria and lactobacilli in the human gut (Broekaert et al., 2011). The prebiotic function is modulated by structural differences in arabinoxylan oligosaccharides, where different lengths and arabinose contents result in different prebiotic effects (Van Craeyveld et al., 2008). In addition to being prebiotics and intermediates in cell wall degradation, cell wall derived oligosaccharides are of biological interest as they have been implicated in growth regulation and signaling pathways (Burton et al., 2010).

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Here, we show that ^{1}H, ^{13}C and ^{1}H–^{13}C NMR spectroscopy provide structural detail for tracking the degradation of cell wall polysaccharides in crude mixtures. In the substrate wheat arabinoxylan, 15 different structural units are resolved and identified as their chemical structures. More than 20 (arabino-)xylooligosaccharide units, most of them near cleavage sites, are directly assigned in oligosaccharide mixtures produced by fungal enzyme preparations. The carbohydrate units can be distinguished using only anomeric signals as the structural reporters (Vliegenthart, Dorland, & Halbeek, 1983), while disregarding other NMR spectral signals. Especially the greater ^{13}C chemical shift dispersion permits the distinction of unsubstituted, mono- and disubstituted xylan units in different structural motifs. Enzyme action on the different structural motifs and limiting enzyme activities in biomass deconstruction can then be directly read out from oligosaccharide structures (Komiyama et al., 2009) and from time-resolved observations of their transformations. Persisting structural motifs of resultant arabinoxylan oligosaccharides can be distinguished in crude carbohydrate mixtures. The approach is used to characterize arabinoxylan degradation with microbial enzyme preparations from *Trichoderma reesei*, *Humicola insolens*, *Aspergillus aculeatus*, *Aspergillus niger*, *Talaromyces emersonii* and *Bacillus subtilis*. Such direct NMR observations have a role to play in facilitating industrial and scientific work streams concerned with the use and understanding of plant cell wall degradation.

2. Materials and methods

2.1. Enzyme preparations

Commercial enzyme preparations from microbial sources were used without further modification or purification. These preparations originated from *T. reesei* (Laminex; Danisco DuPont, Madison, WI, USA), *H. insolens* (Ultraflo L; Novozymes, Bagsværd, Denmark), *A. aculeatus* (Viscozyme; Novozymes, Bagsværd, Denmark), *A. niger* (Finizym; Novozymes, Bagsværd, Denmark), *T. emersonii* (Beerzym BG; Erbslöh, Geisenheim, Germany) and *B. subtilis* (Neutrase; Novozymes, Bagsværd, Denmark).

2.2. Chemicals and samples

Wheat arabinoxylan from Megazyme (Bray, Ireland) was used without further purification or modification. The arabinoxylan was dissolved under gentle heating to a concentration of 0.5% (w/v) in 600 μl of sodium acetate buffer (100 mM, pH 5.3). The buffer was produced as aqueous buffer prior to condensation and redissolution in D_2O (Cambridge Isotope Laboratories, Andover, MA, USA). 2 ml of commercial lager beer were condensed and resuspended in 600 μl D_2O in order to assess the spectral resolution of arabinoxylan fragments from other components of agro-industrial products.

2.3. NMR assays of arabinoxylan degradation

^{1}H NMR spectroscopic assays were conducted at 313 K on an 800 MHz Bruker (Fällanden, Switzerland) Avance spectrometer equipped with a TCI cryoprobe and an 18.7 T magnet (Oxford Magnet Technology, Oxford, UK). A ^{1}H time series of arabinoxylan degradation was acquired by recording 16,384 complex data points during an acquisition time of 1.6 s with a recycle delay of 2 s between the accumulation of 16 transients. In order to assess rapid initial steps of degradation, enzyme preparations were diluted in 100 μl buffer. This solution was taken up into the end of a transfer line attached to a syringe. The transfer line was placed above 500 μl of an arabinoxylan solution in a 5 mm NMR sample tube and the sample was inserted into the NMR magnet and ^{1}H NMR spectroscopic assays were conducted at 313 K on a 600 MHz Bruker DRX spectrometer equipped with a TCI cryoprobe and a 14.1 T magnet. A time series of 8000 ^{1}H NMR spectra was recorded as a pseudo-2D spectrum sampling 2048 complex data points during an acquisition time of 0.85 s, employing a recycle delay of 1 s and the accumulation of 4 transients. Once the pseudo-2D experiment had been started, the enzyme solution was manually injected *via* the transfer line into the sample.

2.4. NMR assignments of arabinoxylan-derived oligosaccharide mixtures

NMR assignments of structural motifs were conducted for intact arabinoxylan and for arabinoxylan oligosaccahrides obtained by partial degradation of arabinoxylan with an *A. aculeatus* preparation (Viscozyme) prior to quenching the degradation by heating to 100 °C for 5 min. Assignment spectra included DQF-COSY (2048 × 512 complex data points with acquisition times of 287 and 72 ms, respectively), NOESY with a mixing time of 200 ms (2048 × 256 complex data points with acquisition times of 307 and 38 ms, respectively), TOCSY with 10 kHz spin lock field during 60 ms (2048 × 512 complex data points sampling 307 and 76 ms, respectively), multiplicity edited ^{1}H–^{13}C HSQC (1024 × 512 complex data points with acquisition times of 153 and 18 ms), ^{1}H–^{13}C HSQC TOCSY (1024 × 256 complex data points sampling 153 and 10 ms), and ^{1}H–^{13}C HMBC (2048 × 256 complex data points with acquisition times of 300 and 6 ms). Some of the ^{1}H assignments could be independently validated by comparison to published assignments of purified arabinoxylan fragments or of intact arabinoxylan at high temperature (340 K) (Gruppen et al., 1992; Hoffmann, Leeflang, de Barse, Kamerling, & Vliegenthart, 1991; Hoffmann, Kamerling, & Vliegenthart, 1992; Kormelink et al., 1993). Spectra were referenced to the αH1 signal of the glucopyranosyl anomer of residual glucose in the Viscozyme preparation at ^{1}H = 5.223 ppm and ^{13}C = 92.990 ppm (Petersen, Hindsgaul, & Meier, 2014). Resultant sequential assignments are summarized in the supplemental Table 1S.

2.5. Data processing and analysis

All spectra were processed with Bruker Topspin 2.1 software using extensive zero filling in all dimensions. The presence and relative abundance of structural motifs were directly assessed from ^{1}H and ^{1}H–^{13}C spectra. The ^{1}H spectrum of arabinofuranosyl-residues in arabinoxylan was reconstructed from assignments of chemical shifts in five different structural motifs containing the arabinofuranosyl-residues. The experimental spectrum of wheat arabnioxylan can be well described as a sum of component spectra as

$$L(\delta) = \sum_{j=1}^{5} \sum_{i} a_j \frac{1}{1 + x_i}$$

where $x_i = [(\delta - \delta_i)/w]^2$. $L(\delta)$ is the Lorentzian function with amplitudes a_j for the five different component structural motifs having i different arabinose anomeric signals at spectral positions δ_i with a line width of $2w$.

3. Results

3.1. Assignment of strucural motifs in intact wheat arabinoxylan at ambient temperature

Arabinoxylan is a hemicellulose with β-1,4-linked D-xylopyranosyl backbone and frequent arabinofuranosyl residue

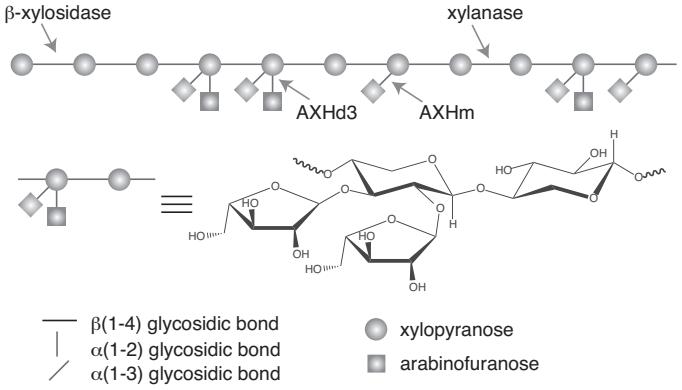

Fig. 1. Schematic depiction of arabinoxylan. Horizontal lines indicate β(1-4)glycosidic bonds between D-xylopyranosyl units (circles), diagonal lines indicate α(1-3)glycosidic bonds of arabinofuranosyl units (squares) to the backbone xylan and vertical lines indicate α(1-3)glycosidic bonds of arabinofuranosyl units to the backbone xylan. Cleavage sites for different enzyme activities are indicated by arrows.

side chains attached to O2 or O3 of the xylan units (Fig. 1) (Scheller & Ulvskov, 2010). In the temperature range that is practical for enzymatic arabinoxylan degradation and that encompasses physiological temperatures, ^{1}H NMR spectra of arabinoxylan yield congested spectra even at high magnetic field (18.7 Tesla, Fig. 2). In addition to the small chemical shift dispersion between carbohydrate units, ^{1}H NMR of carbohydrates suffers from the overlap of the water signal with the carbohydrate spectral region, especially with glucan and xylan fragments. As a consequence, the NMR spectroscopic observation of xylan content in aqueous samples and samples containing residual HOD can be problematic, especially near physiological temperatures.

^{1}H NMR spectra resolve arabinofuranosyl residues in 3- or 2,3-substituted sites (Fig. 2A). These single or disubstituted sites can be located to different structural motifs with diligent analysis of two-dimensional ^{1}H–^{1}H and ^{1}H–^{13}C correlation spectra. Such analysis allows the identification and assignment of 13 chemically distinct arabinofuranosyl residues alone within a chemical shift range of 0.2 ppm at 40 °C (Fig. 2A). These assignments yield chemical shifts for five different major structural motifs in arabinoxylan, specifically (1) arabinofuranose attached to O3 with unsubstituted neighboring xyloses, (2) arabinofuranoses attached to O2 and O3 with unsubstituted neighboring xyloses, (3) two sequential xyloses with arabinofuranoses attached to O2 and O3 and double substituted xylose next to a mono-substituted xylose (4) towards the reducing or (5) towards the non-reducing end. The assignment of anomeric signals in these structural motifs allows the construction of the arabinose spectrum as a weighted sum of component Lorentzian curves for the different structural motifs: In the commercial wheat arabinoxylan substrate, structural motifs containing arabinose branches consisted of 53% mono-substitution at O3, (1), 32% of double-substitution (2) and approximately 5% of structural motives with sequential substituted xyloses (3, 4 and 5; Fig. 2B).

3.2. NMR tracking of arabinoxylan degradation pathways

The complete degradation of substituted xylans is a complex process catalyzed by various glycosidases (O-glycoside hydrolases). Full degradation requires xylanase (EC 3.2.1.8), β-xylosidase (EC 3.2.1.37) and different arabinofuranosidase activities that remove arabinofuranosyl residues from 3- and 2,3-substituted xylose units (EC 3.2.1.55) (Rasmussen, Xu, Sørensen, Nielsen, & Meyer, 2012). Different forms of these arabinoxylan-degrading enzymes exist and they show diverse substrate specificities and activities (Collins, Gerday, & Feller, 2005). The specificities, mechanisms

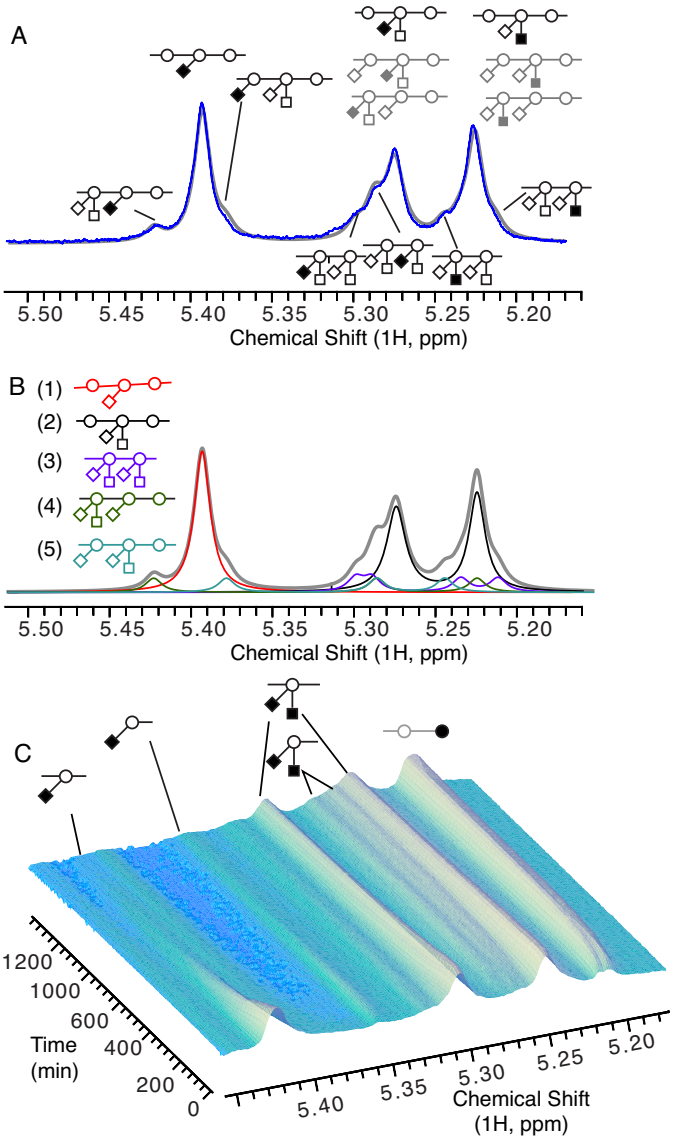

Fig. 2. (A) 800 MHz ^{1}H NMR spectrum of anomeric ^{1}H signals of arabinofuranosyl branches in intact arabinoxylan, showing congested Lorentzian lines of five main contributing structural motifs (blue). Assignments of structural motifs were obtained in intact arabinoxylan using homo- and heteronuclear NMR correlation experiments. The assignments yield simulated spectra of the individual structural motifs (B) that can be used for a weighted superposition (grey) to generate the arabinoxylan spectrum (superposition is overlayed onto the experimental spectrum in A). (C) Injection of an enzyme preparation from *Humicola insolens* (Ultraflo L) into a thermally equilibrated sample of 0.5% (w/v) in sodium acetate buffer (100 mM, pH 5.3) residing in an NMR spectrometer yields partly overlapped reaction progress curves of arabinoxylan degradation at ~seconds temporal resolution. (For interpretation of the references to color in this legend, the reader is referred to the web version of the article.)

and products during arabinoxylan bioconversion can be tracked *in situ* due to the NMR spectroscopic distinction of structural motifs by different, albeit not baseline-separated, signals (Fig. 2C). The detailed observation of chemical transformations in polysaccharide degradation without sample cleanup has previously been demonstrated with α and β glucans, using carefully optimized high-resolution NMR spectra (Beeren, Petersen, Bojstrup, Hindsgaul, & Meier, 2013; Bojstrup, Petersen, Beeren, Hindsgaul, & Meier, 2013; Petersen, Olsen, Beeren, Hindsgaul, & Meier, 2013; Petersen, Hindsgaul, et al., 2014). For tracking degradation pathways of the more complex heteropolymeric arabinoxylan, 100 μl of a commercial enzyme preparation containing hemicellulose

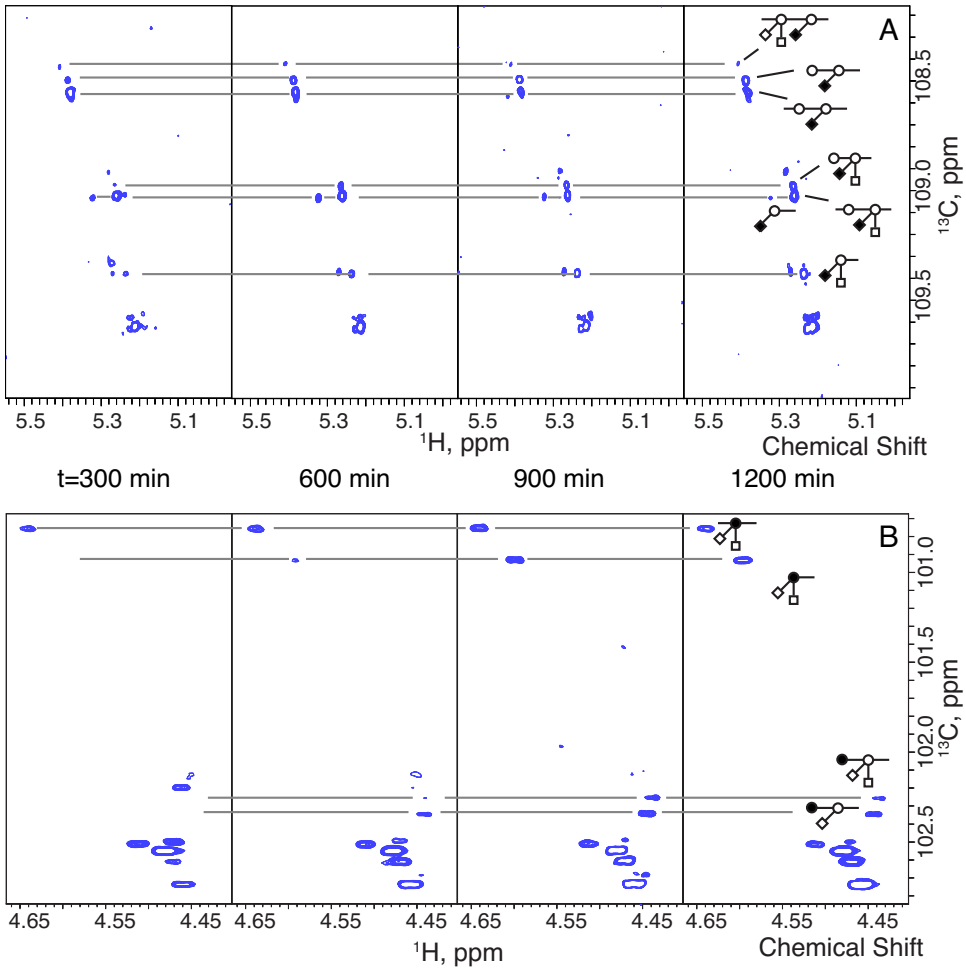

Fig. 3. Time course of arabinoxylan cleavage by a *Humicola insolens* preparation at 313 K. Monitoring cleavage by ^{1}H–^{13}C HSQC resolves cleavage kinetics in the vicinity of single and double branched sites. Spectral regions containing arabinofuranosyl α-anomeric signals are shown in (A), regions containing xylopyranosyl β-anomeric signals are shown in (B). HSQC spectra were recorded in 5 h intervals on the same sample.

activity were taken up into the end of a tube and manually released into a thermally equilibrated 5 mm diameter sample tube containing 500 μl of 0.5% (w/v) wheat arabinoxylan inside a pulsing NMR spectrometer. A time course of the resultant polysaccharide degradation is shown in Fig. 2C.

Homo- and heteronuclear assignment spectra were used to assign structural motifs in the oligomers, specifically their position at or near the cleavage site, in a partially degraded solution of arabinoxylan (Fig. 2C). Using a time series of ^{1}H spectra, cleavage towards the non-reducing end of branched xylopyranosyl residues can thus be determined *in situ*, alongside xylose and arabinose release as well as xylan cleavage, in rapid ^{1}H NMR assays with time resolution of few seconds (given by the ^{1}H acquisition time and the recycle delay). Substantially better spectral resolution of carbohydrate mixtures is achieved using highly resolved ^{1}H–^{13}C HSQC spectra that sample the indirect ^{13}C dimension for more that 150 ms (Beeren et al., 2013; Bojstrup et al., 2013; Petersen, Hindsgaul, et al., 2014).

Fig. 3 shows the ^{1}H–^{13}C HSQC tracking of Ultraflo L-catalyzed arabinoxylan degradation. ^{1}H–^{13}C HSQC spectra with sufficient sampling of the ^{13}C dimension resolve arabinofuranosyl residues by their distance to chain termini (at the non-reducing end, next to the non-reducing end, or farther from the non-reducing end). In addition, distinct β(1-4)xylopyranosyl residues of the xylan backbone emerge upon degradation, and the congestion of the β(1-4)xylopyranosyl anomeric signal with the residual water signal is abolished (Figs. 3 and 4), affording accurate identification of at least 25 quantifiable units in arabinoxylan-fragments other than xylose and arabinose. Rapid fragmentation relative to arabinose release from single and particularly double substituted sites is tracked *in situ*, as oligosaccharides accumulate with branched residues at or near the non-reducing end. Xylan cleavage is more rapid towards the non-reducing end of 3-substituted xylose than towards the non-reducing end of 2,3-substituted residues, as the fraction of 3-substituted residues adjacent to the non-reducing end is higher than the fraction of 2,3-substituted residues adjacent to the non-reducing end (Figs. 3 and 4).

Due to the intrinsically small chemical shift dispersion of carbohydrates, tracking carbohydrate mixtures, for instance in polysaccharide degradation, can benefit from the ~20-fold larger chemical shift dispersion of ^{13}C signals relative to ^{1}H signals. As compared to one-dimensional ^{13}C spectra, ^{1}H–^{13}C HSQC spectra yield a sensitivity gain (*ca* 30-fold) that allows the reasonably fast tracking of enzyme activities in real time even for moderately soluble substrates. This sensitivity gain is fully achieved through extensive sampling of the indirect ^{13}C dimension, which leads to accurate signal measurements. The detection of sharp signals benefits both the signal resolution and the signal to noise ratio. For protonated carbons, projections of HSQC spectra onto the ^{13}C dimension then yield the information of one-dimensional ^{13}C spectra, but at a higher sensitivity, and at improved resolution when using signals from defined regions of interest only. Fig. 5

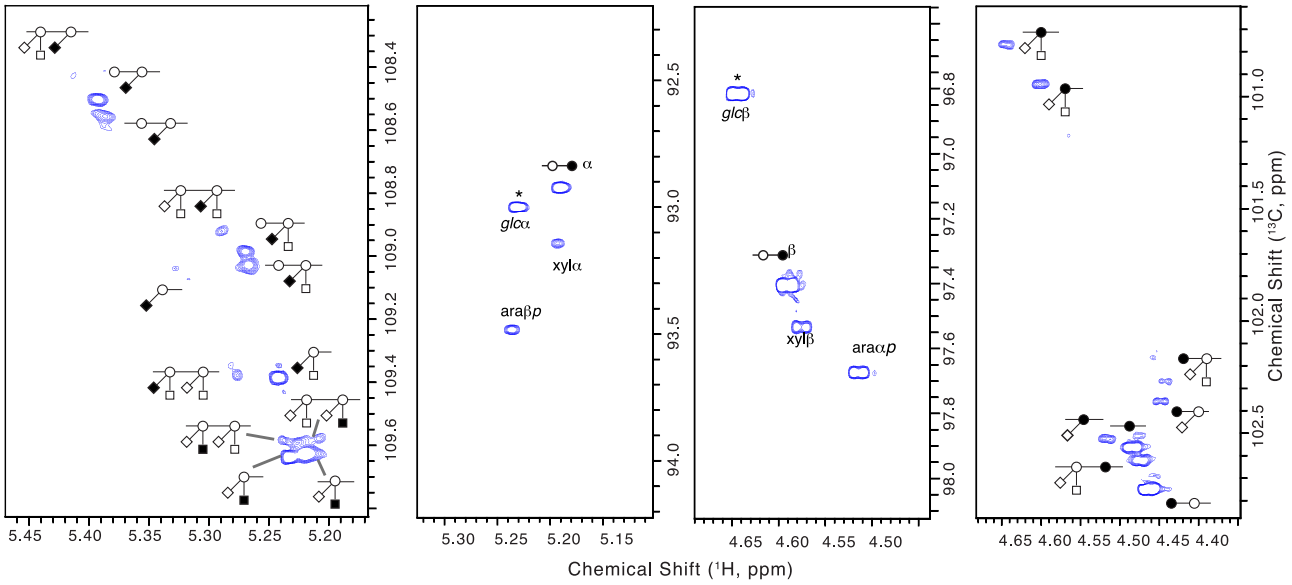

Fig. 4. Regions of a highly resolved $^{1}H–^{13}C$ HSQC spectrum during the cleavage of arabinoxylan with *Aspergillus aculeatus* preparation (Viscozyme). Different spectral regions resolve xylan and arabinose residues and structural motifs of arabinoxylan oligosaccharides that were directly assigned in the carbohydrate mixture. Glucose is identified as a component of the enzyme preparation and is marked with an asterisk.

visualizes the degradation of arabinoxylan using projections from HSQC spectra in the ^{1}H range of 5.4–5.0 ppm. Due to the spectral resolution of cleavage site structures and other structural motifs, oligosaccharide structures present at any given time point can be inferred by readout of the abundance of structural motifs (Fig. 5).

3.3. *In situ spectroscopy of arabinoxylan degradation to probe enzyme activities in crude samples*

The direct visualization of enzymatic polysaccharide hydrolysis by crude enzyme preparations in unpurified samples affords a means of effectively identifying rate-limiting steps in the degradation of the substrate. Such knowledge of bottlenecks in polysaccharide degradation is relevant both for the effective total degradation to monosaccharides and for the production of fragments with desired structural features. Degradation pathways were determined for various enzymatic preparations using commercial arabinoxylan as a substrate. Degradation pathways by six commercial enzyme preparations yield the progression of enzymatic cleavage that is depicted in Fig. 6. The resolution and assignment of the carbohydrates shown in Fig. 4 permits direct readouts of the indicated degradation pathways shown in Fig. 6 using time-resolved NMR on a single sample for each enzyme mixture. While xylanase is a main activity in three of these preparations, arabinoxylan degrading activities were also detected in three preparations that are marketed for other activities than xylan cleavage. Hence, using commercial arabinoxylan as a probe permits the screening of biological preparations for even minor arabinoxylan degrading enzyme activities, by virtue of NMR readout of structural transformations in the arabinoxylan substrate.

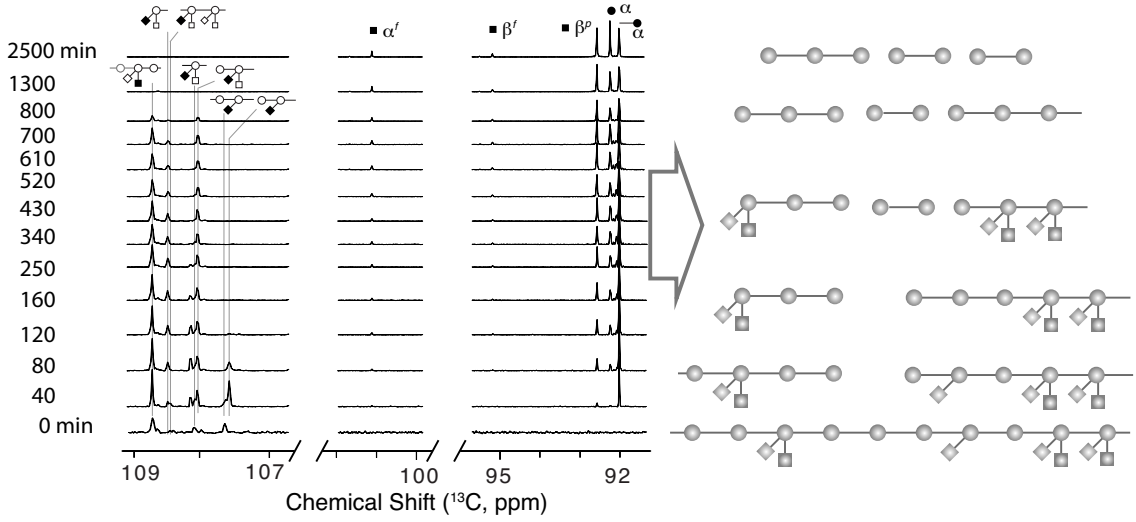

Fig. 5. Degradation pathways of arabinoxylan by Ultraflo L shown with projections of two-dimensional HSQC spectra onto the ^{13}C dimension. The distinction of structural motifs in the ^{13}C spectral dimension yields the pathway of oligosaccharide formation and degradation shown on the right. In particular, the cleavage of arabinose from double substituted xyloses presents a bottleneck, and oligosaccharides with double substituted xyloses at or near the non-reducing end accumulate.

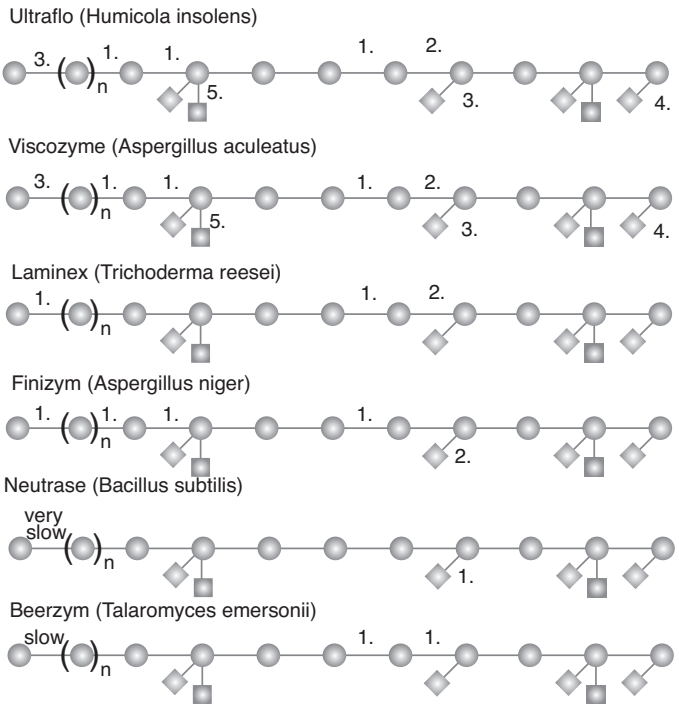

Fig. 6. Different degradation pathways of arabinoxylan by six different microbial enzyme preparations. Cleaved bonds are ranked from the fastest observed cleavage to the slowest observed cleavage. Direct observations of polysaccharide cleavage can be used to probe degrading activities in crude samples.

3.4. HSQC-based resolution of arabinoxylan fragments in agro-industrial products

Fragments of arabinoxylan are omnipresent in cereal-derived foods such as bread, pasta, cookies and beer. As well-tolerated, non-digestible prebiotic carbohydrates, these arabinoxylan fragments have gained importance as food components (Pareyt, Goovaerts, Broekaert, & Delcour, 2011). The increasing health consciousness of consumers therefore necessitates the characterization of oligosaccharides with beneficial properties in food matrices (Swennen, Courtin, & Delcour, 2006). In the brewing industry, arabinoxylan is known to cause filtration problems during production, as arabinoxylan degradation during malting and brewing remains largely incomplete (Petersen, Nilsson, Bojstrup, Hindsgaul, & Meier, 2014). The NMR mapping of structural motifs in arabinoxylan and arabinoxylan oligosaccharide mixtures by high resolution HSQC enables the direct quantitative assessments of the presence of arabinoxylan oligosaccharides in foodstuffs and their degree of degradation. Fig. 7 exemplifies the presence of arabinoxylan fragments in a commercial lager beer sample in comparison to arabinoxylan oligosaccharides derived with commercial enzyme preparations. The example of Fig. 7 shows that the beer arabinoxylan oligosaccharides contain ~10-fold more doubly substituted xylose residues than monosubstituted xyloses, as liberation of arabinose from doubly substituted xyloses seems to be limiting during the malting and brewing processes.

4. Discussion

In the current study, we employ the anomeric $^{1}H–^{13}C$ HSQC spectral region to track – without sample cleanup – specificities, mechanisms and products during the bioconversion of the abundantly available cell wall polysaccharide arabinoxylan. While ^{1}H NMR spectral analyses of carbohydrate mixtures and polysaccharide converting processes yield congested spectra and suffer from solvent overlap, $^{1}H–^{13}C$ HSQC spectra on state-of-the-art NMR equipment provide sufficient sensitivity and resolution even at natural ^{13}C abundance to resolve a plethora of carbohydrate units. In doing so, the approximately 30-fold sensitivity gains of $^{1}H–^{13}C$ HSQC spectra relative to one-dimensional ^{13}C NMR spectra allow

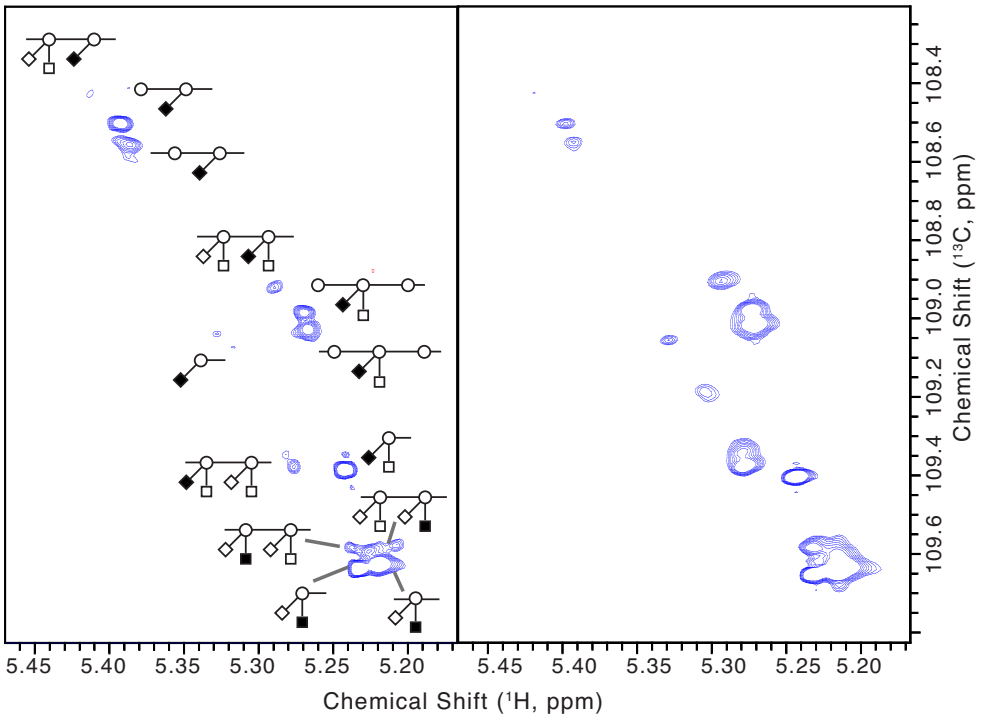

Fig. 7. Identification of arabinoxylan-derived oligosaccharides in cereal-derived foodstuffs by $^{1}H–^{13}C$ HSQC. A spectral region displaying oligosaccharides of arabinoxylan cleavage by Viscozyme is shown on the left. The corresponding spectral region of arabinoxylan-derived fragments in beer (right) shows that $^{1}H–^{13}C$ HSQC entirely resolves arabinoxylan-fragments from other major carbohydrate signals in complex carbohydrate mixtures.

the tracking of cell wall polysaccharide degradation in real-time, as long as the fragments are reasonably soluble. Projections of $^{1}H–^{13}C$ HSQC spectra onto the ^{13}C dimension then can yield both higher sensitivity and improved resolution when using signals from defined regions of interest only. In this manner, the identification of at least 25 units other than xylose and arabinose is achieved in arabinoxylan-fragments during *in situ* assays. The resolution and assignment of these units consequently permit direct readouts of degradation pathways by crude enzyme mixtures (Fig. 6) using time-resolved analysis on a single sample per enzyme mixture, thus facilitating and accelerating the characterization of cell wall polysaccharide degrading activities in crude enzyme mixtures. The same considerations apply in assays of the liberation of oligosaccharides and changes to cell wall chemistry during hydrothermal rather than enzymatic treatment of biomass (Kabel et al., 2002). Such assays will be feasible in the absence of product fractionation, as was recently shown using $^{1}H–^{13}C$ HSQC spectra of cell wall preparations even without extensive sampling of the ^{13}C dimension (Yelle et al., 2013).

The rapid and unambiguous spectroscopic characterization of cleaving activities and resultant oligosaccharide structures has a role to play in analyzing the biotechnological valorization of biomass and in characterizing glycoside hydrolase activities of cell wall degrading microorganisms that are central to the carbon cycle and to pathogen invasion of plants (Gilbert, Knox, & Boraston, 2013). Functional assays with chemical detail should complement the increasingly available genome data of cell wall degrading microorganisms (Martinez et al., 2004, 2008; Ohm et al., 2010) and the increasingly detailed structural understanding of plant cell wall recognition by microbial enzymes (Gilbert et al., 2013). As the major component of dietary fiber in cereal grains, arabinoxylan fragments also constitute a considerable fraction of our diet (Lu, Walker, Muir, Mascara, & O'Dea, 2000). The reported health benefits of arabinoxylan-derived oligosaccharides make the simple characterization of these oligosaccharides in complex matrices desirable, especially as the prebiotic properties depend on structural details in the fragments. Hence, the rapid detection of structural detail in the oligosaccharides of food products (Fig. 7) bears promise for supporting structure–function relationships for functional food components (Broekaert et al., 2011; Van Craeyveld et al., 2008).

Several agro-industrial residues contain on the order of 20–40% hemicelluloses (Saha, 2003), with xylan-type polysaccharides being the main constituents of hemicelluloses and the second most abundant natural polysaccharide (Collins et al., 2005). We therefore expect that rapid high-resolution methods providing atomic detail of polysaccharides and mixtures of their fragments will support the increasingly effective usage of biomass. $^{1}H–^{13}C$ NMR provides structural detail to complement more commonly used chromatographic analyses of xylan degradation (Ishii et al., 2008). Alternatively, it is conceivable to retrieve size information concurrently to HSQC analyses from the self-diffusion properties of the $^{1}H–^{13}C$ HSQC NMR signals (Vitorge & Jeanneat, 2006).

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