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Structural studies on water-soluble arabinoxylans in rye grain using enzymatic hydrolysis

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The major water-soluble arabinoxylan fraction from rye grain, containing 4-linked β-D-xylopyranosyl residues of which about 43% were substituted solely at O-3 and 7% at both O-2 and O-3 with terminal α-L-arabinofuranosyl units, was hydrolysed to different extents using semi-purified xylanase from *Trichoderma reesei*. Products were fractionated on Biogel P-2 and structurally elucidated by sugar, methylation and high-field ^{1}H-NMR analysis. Moderate hydrolysis released arabinose, xylose, xylobiose, xylotriose and xylotetraose together with xylo-oligosaccharides (DP ⩾ 4) in which one or more of the residues were substituted at O-3 with a terminal arabinose unit. The xylose residues substituted with arabinose units at both O-2 and O-3 became enriched in the remaining polymeric fraction. Extensive hydrolysis with the enzyme released arabinose, xylose and xylobiose as major products together with small amounts of two oligosaccharides and a polymeric fraction. One of the oligosaccharides was identified as xylotriose in which the non-reducing end was substituted at O-2 and O-3 with terminal arabinose units and the other as xylotetraose in which one of the interjacent residues was substituted with arabinose units in the same way. The polymeric fraction contained a main chain of 4-linked xylose residues in which 60–70% of the residues were substituted at both O-2 and O-3 with arabinose units.

The semi-purified enzyme contained xylanase and arabinosidase activities which rapidly degraded un- and mono-substituted xylose residues while the degradation of double-substituted xylose residues was much slower. The results show that the mono- and double-substituted xylose residues were present in different polymers or different regions of the same polymer.

INTRODUCTION

Arabinoxylans are the predominant components of rye endosperm cell walls (Fincher & Stone, 1986) and the major water-soluble arabinoxylan fraction has been shown to contain a main chain of 4-linked β-D-xylopyranosyl residues of which about 50% are substituted at O-3 with terminal α-L-arabinofuranosyl residues (Aspinall *et al.*, 1960; Bengtsson & Åman, 1990). The branched xylose residues are present as isolated units or small blocks of predominantly two residues (Åman & Bengtsson, 1991). Some of the xylose units are double-substituted at O-2 and O-3, also by terminal α-L-arabinofuranosyl residues (Fincher & Stone, 1986). Variable contents of double-branched residues have been found in different water-soluble fractions of rye grain (Bengtsson & Åman, 1990) as well as flour, dough and bread of wheat (Westerlund *et al.*, 1990), indicating that a separate polymer or region of a polymer is present in cereals with a high content of double-branched xylose residues. However, there is no conclusive evidence for such a structure.

Many different microorganisms produce arabinoxylan degrading enzymes. These are classified according to their substrate specificity and include endo-xylanases (EC 3.2.1.8), hydrolysing 4-linked β-D-xylopyranosyl residues, and β-xylosidases (EC 3.2.1.37), catalysing the hydrolysis of xylo-oligosaccharides (Dekker & Richards, 1976; Puls *et al.*, 1988; Deshpande *et al.*, 1989). The presence of enzymes responsible for cleavage of side groups, e.g. α-L-arabinofuranosidases (EC 3.2.1.55) catalysing the hydrolysis of non-reducing terminal

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arabinose units, significantly enhances the hydrolytic action of many xylanases and the lack of such an enzyme activity may result in an accumulation of oligomeric degradation products (Puls *et al.*, 1988). Xylanases have been reported to require a binding site of 3–5 xylose residues (Kubačková *et al.*, 1979; Biely *et al.*, 1981; Comtat & Joseleau, 1981), often with a spatial requirement of at least two adjacent unsubstituted residues (Matheson & McCleary, 1985).

Fragmentation of arabinoxylans is generally accomplished by acid or enzymatic hydrolysis. Since arabinose units generally are furanosidically linked they are particularly sensitive to acid hydrolysis and can thus be selectively removed. Mixed arabinose–xylose oligosaccharides are mainly prepared under the action of xylanases (Bishop & Whitaker, 1955) and are often purified from other hydrolysis products, such as arabinose, xylose, xylobiose and xylo-oligosaccharides, by different types of chromatography. Interestingly, after incubation with xylanases from different sources, arabinose–xylose oligosaccharides have been identified with terminal arabinofuranosyl residues linked solely to O-3 of the reducing, interjacent or non-reducing unit of the xylo-oligosaccharide (McCleary & Matheson, 1986). To our knowledge, no oligosaccharides containing double-branched xylose residues have been identified after enzymatic hydrolysis of cereal arabinoxylans.

In the present investigation water-soluble arabinoxylans from rye were fragmented with a semi-purified xylanase. Low-molecular weight as well as polymeric products were isolated and structurally elucidated in order to gain further information on the structure of rye arabinoxylans, especially on the occurrence and distribution of double-substituted xylose residues.

MATERIAL AND METHODS

Material

Winter rye (cv. Kungs 2) was harvested during 1986 in the south of Sweden near Landskrona. The grain was dried and stored as whole-kernels. Prior to analysis a representative sample (500 g) of the grain (94% dry matter) was ground to pass a 0·5 mm screen.

General methods

Chemicals used were of analytical grade. All analyses were carried out at least in duplicate and are reported on a dry matter basis. The polysaccharide residue content in isolated fractions (1–2 mg) was determined after hydrolysis in 1 M trifluoroacetic acid (121°C, 90 min). The sugars formed were reduced, acetylated and analysed by GLC on a CP-Sil 88 capillary column (Theander & Westerlund, 1986).

For methylation analysis, isolated fractions (1 mg) of polysaccharides or oligosaccharides were reduced with sodium borohydride, dried in vacuum, methylated by a modified Hakamori procedure, hydrolysed with trifluoroacetic acid, reduced and acetylated as described by Harris *et al.* (1984). The identities of the *O*-acetylated, *O*-methylated alditols were established by their GLC retention times on a DB-1 capillary column (Oakley *et al.*, 1985) and by their e.i. mass spectra (Björndal *et al.*, 1970) using a Finnegan 4021 mass spectrometer at 70 eV and an Incos 2000 data system.

The ^{1}H-NMR spectra (400 MHz) were recorded at 85°C on a Varian VXR 400 instrument. About 1000 pulses were given, pulse repetition time was 3·75 s and r.f. pulse angle 45°. The dried fractions (1–4 mg) were dissolved in D_2O (0·7 ml) and sodium 3-trimethylsilyltetradeuteriopropionate was used as reference.

Isolation of the water-soluble arabinoxylan fraction

Rye meal (300 g) was refluxed in 90% aqueous ethanol, the insoluble residue extracted with water at 40°C and soluble components precipitated with ammonium sulphate as previously described (Bengtsson & Åman, 1990). The precipitate was dissolved in hot water (500 ml) and treated with a thermostable α-amylase (Termamyl 60 L, 10 ml; Novo A/S, Denmark) for 30 min at 95°C. After cooling to room temperature the crude arabinoxylan was precipitated and washed with aqueous ethanol and thereafter suspended in hot water, dialysed against distilled water and freeze-dried (Bengtsson & Åman, 1990).

The isolated crude arabinoxylan (2·2 g) was dissolved in 200 ml water using an Ultra-turrax and the small amounts of insoluble materials were removed by centrifugation. The soluble arabinoxylans in the supernatant were applied on a column (5 × 60 cm) of DEAE-SS cellulose (Serva Feinbiochemica, Germany) prepared according to Neukom *et al.* (1960) and eluted with water (2000 ml). Fractions (25 ml) were collected and their carbohydrate content determined by the phenol-sulphuric acid method (Dubois *et al.*, 1956). All carbohydrate-containing materials were pooled, dialysed (MW cut off 12 000) against deionized water and freeze-dried, yielding the main water-soluble arabinoxylan fraction (AX; 1·2 g).

Purification of β-D-xylanase

A crude xylanase (Econase, EP 1262-P, 60000 XNU/g) from *Trichoderma reesei* was a gift from Alko Ltd (Finland). The enzyme (400 mg) was suspended in 0·005 M sodium phosphate buffer (10 ml, pH 6·8) and applied on a DEAE-Sephacel (Pharmacia, Sweden) column (2·5 × 25 cm) which was eluted with the same buffer (0·5 ml/min) in a Pharmacia FPLC system (GrootWassink *et al.*, 1989). Fractions (5 ml) were collected and the xylanase activity in the fractions

determined using an oat spelt xylan (Sigma No. X-0376, USA) as substrate and the reducing sugar assay (Somogyi, 1952; Kanda *et al.*, 1985; McCleary & Glennie-Holmes, 1985). All fractions containing xylanase activity (7–18) were pooled, dialysed for 2 days against deionized water at 4°C and freeze-dried, giving the semi-purified xylanase (160 mg).

Enzymatic hydrolysis

Viscosity was determined in a Bohlin Visco 88 instrument (Bohlin Reologi AB, Sweden) fitted with a closed concentric cylinder system (C 14). The main water-soluble arabinoxylan (60 mg) was dissolved in 6 ml sodium acetate buffer (0·1 M, pH 5·0) and equilibrated at 20°C in the viscometer. Thereafter the semi-purified xylanase (0·17 mg) was added and the change in viscosity and increase in reducing end-groups were followed (Fig. 1).

The main water-soluble arabinoxylan (100 mg) was dissolved in 10 ml sodium acetate buffer (0·1 M, pH 5·0) and then hydrolysed with different amounts of the semi-purified xylanase (0·25, 2·50 and 10·00 mg) for 26 h at 20°C. The hydrolysed materials were boiled for 10 min and, after cooling to room temperature, concentrated to about 1 ml in a vacuum centrifuge. The pellet, containing less than 2% of the carbohydrates, was removed and the supernatant applied on a Biogel P-2 column (2 × 90 cm; Bio-Rad Laboratories, Australia) and eluted with water (0·25 ml/min) in the FPLC-system. The separation was followed by refractive index and fractions (5 ml) were collected (Fig. 2), freeze-dried and stored in vacuum.

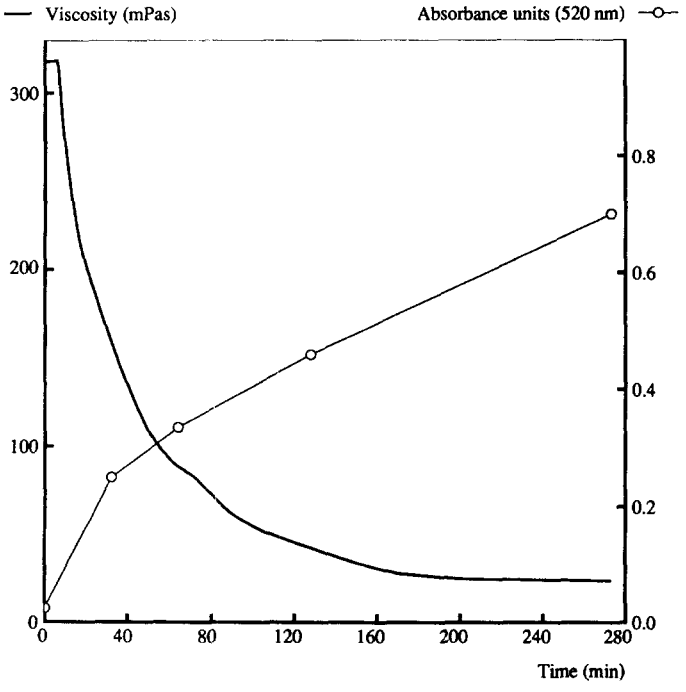


Fig. 1. Decrease in viscosity and increase in reducing end-groups during hydrolysis of the main water-soluble arabinoxylan with semi-purified xylanase (for experimental details see Material and Methods).

RESULTS AND DISCUSSION

The main water-soluble arabinoxylan in rye grain was isolated essentially as described previously (Bengtsson & Åman, 1990). Sugar, methylation and ^{1}H-NMR analysis revealed that the major fraction (AX; 0·4% of dry grain), eluted with water from a DEAE-SS cellulose column, contained a 4-linked main chain with 4-, 3,4-, and 2,3,4-linked β-D-xylopyranosyl residues in a molar ratio of 1·0:0·9:0·1. Terminal α-L-arabinofuranosyl residues were again found to be the only side-units and no acetyl or phenolic substituents could be detected in the ^{1}H-NMR spectrum of the polysaccharide fraction. Previously it has been found that ferulic acid is bound to arabinose residues of pentosans in wheat bran (Smith & Hartley, 1983). The content of double-branched (2,3,4-linked) xylose residues was signifi-

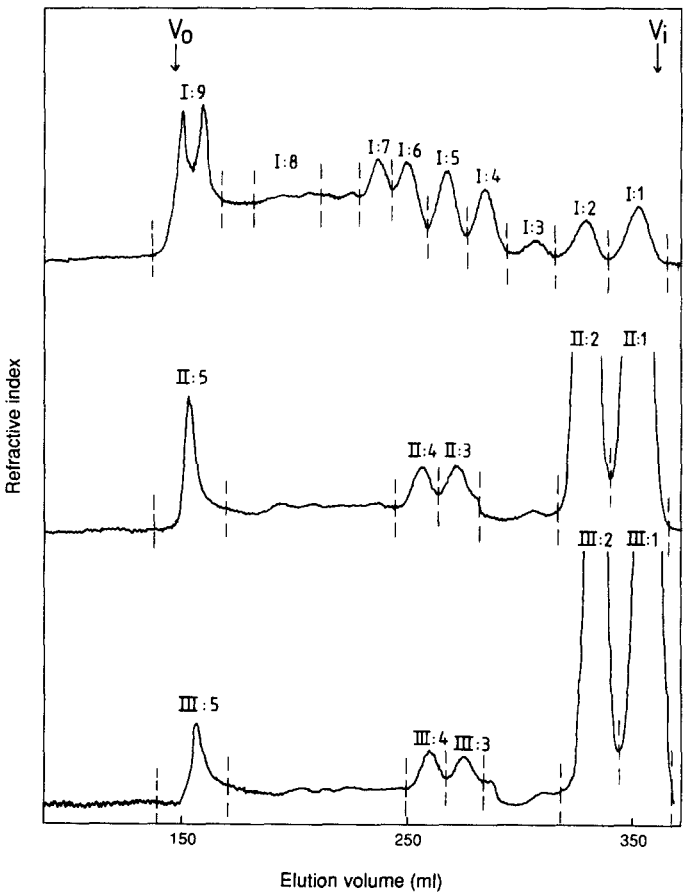


Fig. 2. Gel filtration on Biogel P-2 of products obtained after enzymatic hydrolysis of the main water-soluble rye arabinoxylan using different amounts of semi-purified β-D-xylanase (I = 0·25 mg, II = 2·50 mg and III = 10·00 mg) for 26 h at 20°C. The separation was followed by changes in refractive index.

cantly higher than previously reported (Bengtsson & Åman, 1990) but this may be due to the large-scale fractionation on DEAE-SS cellulose used in this study.

β-D-Xylanase from *Trichoderma reesei* was purified on DEAE-Sephacel. The non-bound fraction, eluted with 0·005 M sodium phosphate buffer and containing β-D-xylanase activity as measured by the reducing sugar assay with oat spelt xylan as substrate, was isolated. In previous studies similar purifications have produced relatively pure β-D-xylanases with low α-L-arabinofuranosidase activity (Gibson & McCleary, 1987; GrootWassink *et al.*, 1989). Incubation of rye arabinoxylan with the semi-purified enzyme showed that the decrease in viscosity was more rapid than the increase in reducing end-groups (Fig. 1), indicating a high endo-xylanase activity (Deshpande *et al.*, 1989).

The water-soluble arabinoxylan was hydrolysed with different amounts of this semi-purified enzyme and the products fractionated on Biogel P-2 (Fig. 2). The structure of oligosaccharides in isolated fractions (see Table 1) was determined by sugar, methylation and ^{1}H-NMR analysis. Hydrolysis with low amounts of enzyme (I = 0·25 mg) revealed a complex mixture of components. Fraction I:1 contained free arabinose and xylose, fraction I:2 xylobiose and fraction I:3 xylotriose. Fraction I:4 most likely contained traces of xylotetraose as revealed by the small amounts of 4-linked interjacent xylopyranosyl residues in the methylation and ^{1}H-NMR analysis (see Fig. 3). The predominant component (>90%) in this fraction, however, was a tetrasaccharide containing arabinose and xylose in a molar ratio of 1:3. Methylation analysis of the reduced oligosaccharide identified one terminal arabinofuranosyl residue linked to O-3 of the middle xylopyranosyl residue in xylotriose. ^{1}H-NMR spectra were in accordance with this structure (Fig. 3). This oligosaccharide has previously been isolated from wheat arabinoxylan after enzymatic hydrolysis (Goldschmid & Perlin, 1963). Fraction I:5 contained a pentasaccharide with arabinose and xylose in a molar ratio of 1:4. Methylation analysis of the reduced oligosaccharide identified xylotetraose with one of the interjacent residues substituted at O-3 with a terminal α-L-arabinofuranosyl residue. Fractions I:6–8 all contained higher oligosaccharides, with 4- and 3,4-linked xylopyranosyl residues and terminal arabinofuranosyl residues as dominating constituents.

When the water-soluble arabinoxylan (AX) was incubated with higher amounts of enzyme (II = 2·5 or

Table 1. ^{1}H-NMR chemical shifts of anomeric protons in oligosaccharides isolated after enzymatic hydrolysis of water-soluble rye arabinoxylan

Oligosaccharide structure[a]	Fraction[b]	Chemical shift (ppm)					
		X_a	X_b	X_c	X_d	A_a	A_b
$X_a \rightarrow 4X_b$	I:2, II:2 and III:2	4·47	4·60(β)[c] 5·19(α)[c]				
$X_a \rightarrow 4X_b \rightarrow 4X_c$	I:3	4·45	4·50	4·60(β) 5·19(α)			
$X_a \rightarrow 4X_b \rightarrow 4X_c$ 3 ↑ A_a	I:4	4·46	4·54	4·60(β) 5·19(α)		5·40	
$X_a \rightarrow 4X_b \rightarrow 4X_c \rightarrow 4X_d$ 3 ↑ A_a	I:5	4·46	4·54[e]	4·50[e]	4·60(β) 5·19(α)	5·40	
$X_a \rightarrow 4X_b \rightarrow 4X_c$ 3 2 ↑ ↑ A_a A_b	II:3 and III:3	4·63	4·50	4·60(β) 5·19(α)		5·27[d]	5·26[d]
$X_a \rightarrow 4X_b \rightarrow 4X_c \rightarrow 4X_d$ 3 2 ↑ ↑ A_a A_b	II:4 and III:4	4·45	4·68[e]	4·50[e]	4·60(β) 5·19(α)	5·30[d]	5·23[d]

[a] X = β-D-xylopyranosyl and A = α-L-arabinofuranosyl; X_a—X_b is equivalent to β-D-xylopyranosyl-(1→4)-D-xylopyranosyl and so on.
[b] The fraction in which the oligosaccharide was identified (see Fig. 2).
[c] Two anomeric forms at the reducing end.
[d] Assignment may be reversed.
[e] Substitution may be on the other interjacent residue.

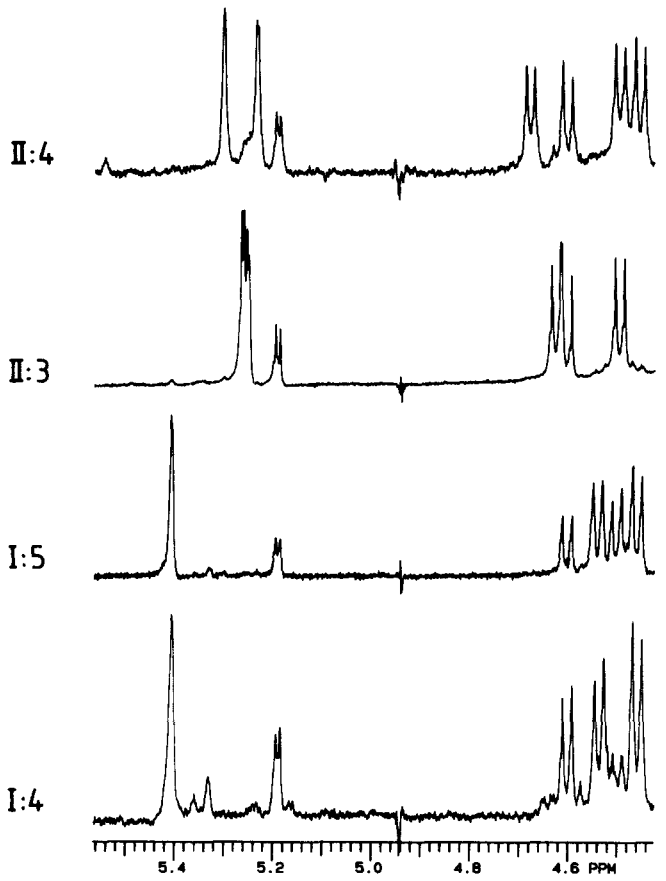


Fig. 3. ^{1}H-NMR spectra of the anomeric region of some low-molecular weight fractions (I:4, I:5, II:3, II:4) obtained after enzymatic hydrolysis with different amounts of semi-purified β-D-xylanase. Structural features of main oligosaccharide in the fraction are given in Table 1.

III = 10·0 mg) less complex profiles of products were obtained after fractionation on Biogel P-2 (Fig. 2). The two chromatograms were similar, with arabinose and xylose (fractions II:1 and III:1) and xylobiose (fractions II:2 and III:2) as dominating components. In fractions II:3 and III:3, a pentasaccharide containing arabinose and xylose in a molar ratio of 2:3 was identified. Methylation analysis of the reduced oligosaccharide revealed two terminal arabinofuranosyl residues, one 2,3- and one 4-linked xylopyranosyl residue and one 4-linked xylose residue at the reducing end. These results clearly identified the oligosaccharide as xylotriose, substituted at O-2 and O-3 of the non-reducing end with two terminal α-L-arabinofuranosyl residues. ^{1}H-NMR spectra of the oligosaccharide (Fig. 3) were in accordance with the proposed structure. Double-substitution of xylose residues have previously been detected in rye, wheat and barley (Aspinall & Sturgeon, 1957; Medcalf & Gilles, 1968; Bacic & Stone, 1981) but little is known about the polysaccharide structures containing this residue.

Fractions II:4 and III:4 contained a hexasaccharide with arabinose and xylose in a molar ratio of 2:4. Methylation analysis of the reduced oligosaccharide revealed two terminal arabinofuranosyl residues together with the expected residues from xylotetraose in which one of the interjacent residues was branched at position 2 and 3 with the two arabinose residues.

The ^{1}H-NMR chemical shifts of anomeric protons in isolated xylobiose and oligosaccharides are listed in Table 1. All compounds contained an unbranched xylose residue at the reducing end as revealed by the resonances at 4·60, (β-pyranosyl) and 5·19 ppm (α-pyranosyl) (Andersson *et al.*, 1990). Resonances at 4·45–4·47 ppm revealed unbranched xylose residues at the non-reducing end and those at 4·50 ppm interjacent unbranched xylose residues, in accordance with the literature (Capon & Thacker, 1964; De Silva *et al.*, 1986). The chemical shift of the anomeric proton of an interjacent xylose residue substituted at O-3 with a terminal arabinofuranosyl residue was moved about 0·04 ppm downfield (4·54 ppm) compared to the corresponding unbranched xylose residue and the shift of the attached arabinose substituent appeared at 5·40 ppm, in accordance with the corresponding residues in the polymeric arabinoxylan (Bengtsson & Åman, 1990). The anomeric proton in an interjacent xylose residue substituted at both O-2 and O-3 with terminal α-arabinofuranosyl residues was moved about 0·18 ppm (4·68 ppm) downfield compared to the corresponding unbranched xylose residue and the anomeric protons of the attached arabinose substituents appeared at 5·23 and 5·30 ppm, also in accordance with the corresponding residues in the arabinoxylan polymer (Bengtsson & Åman, 1990). Similar double-substitution at the non-reducing end of a xylose residue also moved the chemical shift of the anomeric proton downfield in the same order (0·17 ppm) compared to the corresponding unbranched xylose residue. It is evident that high-field ^{1}H-NMR spectra, and especially resonances of the anomeric protons, are very useful for structural elucidation as well as quantitative analysis of purified and complex mixtures of oligosaccharides and polymeric fractions of arabinoxylans.

From Fig. 2 it is apparent that a small fraction of the main water-soluble arabinoxylan was not easily degraded by the enzyme and consequently appeared as a polymeric fraction, even after extensive hydrolysis (II:5 and III:5). It is also seen, however, that this polymeric fraction was slowly degraded to low-molecular weight components, since the relative yield of this fraction diminished with increasing extent of hydrolysis (compare I:9, II:5 and III:5). Sugar analysis of the polymeric fractions revealed arabinose and xylose as the dominating sugar residues with an increasing relative content of arabinose residues (from 35 to about 60%) from AX to III:5. Methylation analysis (Table 2) showed that fraction I:9, which included a partially resolved more low-molecular weight peak, contained more terminal arabinofuranosyl, terminal

Table 2. Glycosyl linkage composition of the polymeric fractions[a] (I:9, II:5 and III:5) isolated after enzymatic hydrolysis of water-soluble rye arabinoxylan (AX)

Residue	Position of *O*-methyl groups	T^b	Product composition (mol %)				Structural unit deduced
			AX	I:9	II:5	III:5	
Ara	2,3,5	389	35	44	60	57	Ara(*f*)→
Xyl	2,3,4	432	—	2	—	—	Xyl(*p*)→
Xyl	2,3	540	32	18	10	8	→4-Xyl(*p*)→
Xyl	2	640	28	23	—	—	→3,4-Xyl(*p*)→
Xyl	—	731	4	13	30	35	→2,3,4-Xyl(*p*)→

[a] Fractionated on Biogel P-2 as shown in Fig. 2.
[b] Retention time (×1000) of the corresponding alditol acetate relative to that of *myo*-inositolhexa-acetate (see Material and Methods).

xylopyranosyl and 2,3,4-linked xylopyranosyl residues and less 4- and 3,4-linked xylopyranosyl residues compared to the isolated water-soluble arabinoxylan (AX). These results are in accordance with a major enzymatic hydrolysis of the unbranched and branched (at the 3-position) xylopyranosyl residues. The polymeric fractions isolated after the more extensive hydrolysis (II:5 and III:5) contained mainly terminal arabinofuranosyl and 2,3,4-linked xylopyranosyl residues together with about 10% of 4-linked xylopyranosyl residues. It is evident that all branched (at the 3-position) xylopyranosyl residues had disappeared from the polymeric fraction, that the double-branched xylopyranosyl residues were very resistant to the enzyme, and that no 2-linked xylose residues were detected during the hydrolysis. The fact that no terminal xylopyranosyl residues could be detected in these fractions showed that they had high molecular weights.

^{1}H-NMR spectra of anomeric protons in the isolated polymeric fractions revealed the same changes in composition as the methylation analysis (Fig. 4). In the unhydrolysed polymer resonances were identified, as described previously (Smith & Hartley, 1983; Bengtsson & Åman, 1990), as arabinofuranosyl residues linked to O-3 of xylose units (5·4 ppm), terminal arabinofuranosyl residues linked to both O-2 and O-3 of double-branched xylose units (5·2 and 5·3 ppm), 2,3,4-linked xylose residues (4·68 ppm), 3,4-linked xylose residues (~4·5 ppm, left part) and 4-linked xylose residues (~4·5 ppm, right part). Hydrolysis with relatively low amounts of enzyme resulted in a polymeric fraction I:9 enriched in terminal arabinofuranosyl residues linked to both O-2 and O-3 of double-branched xylose units (multiplets at 5·2 and 5·3 ppm) and double-branched xylose units (4·57 and 4·67 ppm) compared to the unhydrolysed polymer. The signal around 4·6 ppm also contained some of the resonance from unbranched xylopyranosyl units appearing at the reducing end (β-form; α-form seen at 5·19 ppm). Extensive enzymatic hydrolysis

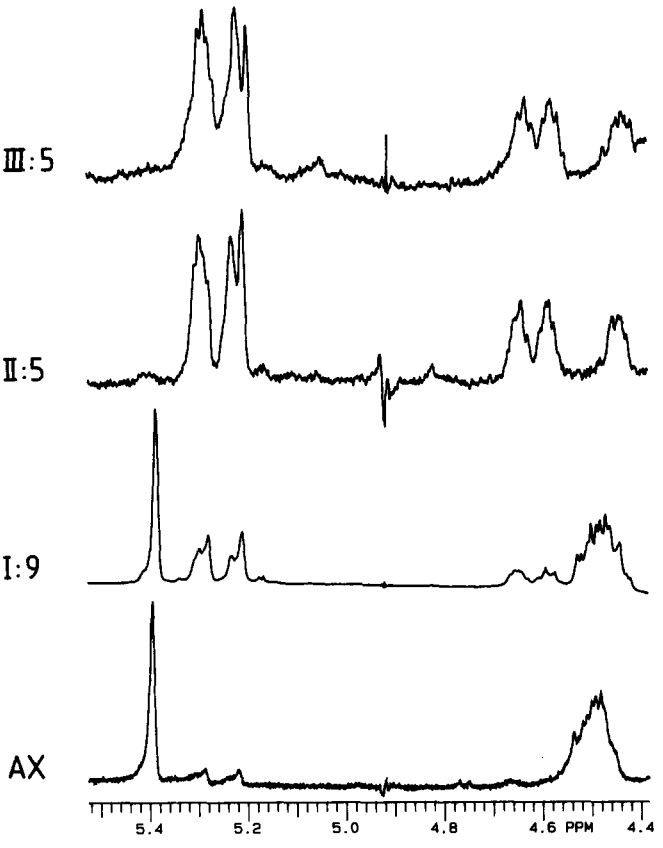
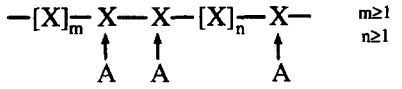


Fig. 4. ^{1}H-NMR spectra of the anomeric region of the main water-soluble rye arabinoxylan (AX) and polymeric fractions (I:9, II:5, III:5) obtained after enzymatic hydrolysis with different amounts of semi-purified β-D-xylanase.

resulted in polymeric fractions (II:5 and III:5) further enriched in double-branched xylose units and the attached terminal arabinofuranosyl residues. Resonances from 4-linked xylose units (4·47 ppm) were also identified. It is notable, however, that the signals for anomeric protons from the 3,4-linked xylose units had disappeared as well as corresponding signals from attached arabinose residues (5·4 ppm).

Arabinoxylan I



Arabinoxylan II

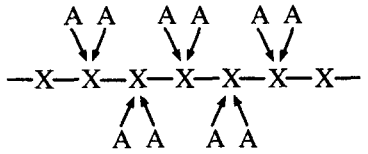


Fig. 5. Structural features of arabinoxylan I and II in rye grain. A = terminal α-L-arabinofuranosyl and X = 4-linked β-D-xylopyranosyl residues.

The anomeric resonances from the terminal arabinofuranosyl residues linked to the double-branched xylose residues appeared as doublets or multiplets in spectra of the isolated polymeric fractions (Fig. 4). The corresponding 2,3,4-linked xylopyranosyl residues also appeared as two multiplets. These multiple peaks were probably a result of different influences of neighbouring sugar residues, unbranched or double-branched xylose residues in the main chain, as have previously been found in other polysaccharides (Grasdalen *et al.*, 1988).

The results presented have shown that the main water-soluble arabinoxylan fraction contained at least two polymers or two fractions of a polymer. The major fraction (arabinoxylan I, Fig. 5) had, as previously described, a main chain of 4- and 3,4-linked xylopyranosyl residues with terminal arabinose substituents at O-3 (Bengtsson & Åman, 1990; Åman & Bengtsson, 1991). This fraction was rapidly degraded by the semi-purified enzyme containing appropriate endo-xylanase and arabinofuranosidase activities (Shibuya *et al.*, 1983). The minor fraction (arabinoxylan II, Fig. 5) contained mainly 2,3,4-linked xylopyranosyl residues with terminal arabinose substituents at both O-2 and O-3. This fraction was very resistant to hydrolysis with the enzyme employed, although a slow but notable degradation was evident. This degradation was probably initiated by the release of both arabinose substituents on one of the xylose residues, introducing unbranched xylose residues in the main chain. When sufficient unbranched residues were formed, the main chain was hydrolysed, releasing the oligosaccharides containing double-branched xylose residues.

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