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Short Communication

Evidence of intermolecular interactions of β-glucans and arabinoxylans

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β-Glucans and arabinoxylans are the two major non-starch polysaccharides (NSP) in barley (Henry, 1987). β-Glucans are linear polymers of glucose residues linked via a mixture of β-(1 → 3) and β-(1 → 4) linkages and are usually considered as chains of β-(1 → 3) linked cellotriosyl and cellotetraosyl units arranged randomly (Buliga, Brant & Fincher, 1986). Scientific interest in barley β-glucans stems partly from the problems they cause in brewing and animal feed industries and partly from the benefits they may offer to human diets (Stone & Clarke, 1992). β-Glucans are thought to be associated with filtration difficulties of worts and beers, decreased brewhouse yield, formation of haze and precipitate in beer, and low nutrient adsorption when barley is fed to animals. Otherwise, β-glucans as constituents of dietary fiber are associated with health benefits and appear to be responsible for cholesterol lowering properties of some cereals. Arabinoxylans consist of linear chain backbones of β-(1 → 4) linked xylose residues to which single arabinose residues are attached through C-2 and/or C-3 atoms of the xylose units. Although barley arabinoxylans also have the potential to form viscous solutions and contribute to processing problems normally connected with β-glucans, they have not been extensively studied (Fincher & Stone, 1986; Vietor, Angelino & Voragen, 1993). Even less explored have been the possible interactions between barley β-glucans and arabinoxylans. Although non-covalent interactions are considered a plausible explanation for partial insolubility of barley NSP, neither the molecular characteristics required for such interactions, nor actual interactions have yet been demonstrated experimentally. This communication provides some evidence of interactions between the two polymers.

Based on initial differences in solubility, β-glucans and arabinoxylans can be classified into water-extractable and alkali-extractable polymers. Using sequential extraction with water (40 and 65°C), $Ba(OH)_2$, water, and NaOH, various fractions of β-glucans and/or arabinoxylans were obtained from barley (Izydorczyk, Macri & MacGregor, 1998a,b). The NaOH fraction, the least soluble fraction of barley NSP, contained a mixture of arabinoxylans (58.2%) and β-glucans (41.0%), as estimated from the monosaccharide composition of this fraction (Table 1). Separation of β-glucans from arabinoxylans in mixed solutions can usually be accomplished by adjusting the saturation level of $(NH_4)_2SO_4$ in the solution to 25–30%, which causes precipitation of β-glucans; arabinoxylans, which require much higher saturation of $(NH)_4SO_4$ to achieve 'salting out', stay in the solution. However, our attempt to separate these two polymers in the NaOH extracted fraction failed to produce the expected results. The subfraction obtained at the lowest saturation level of $(NH_4)_2SO_4$ (30%) contained, in addition to β-glucans, approximately 21% of arabinoxylans (Table 1). The presence of arabinoxylans in the subfraction was unusual and appears to indicate some type of interaction between the two polymers. The existence of covalent linkages between the polymers may be excluded as they eluted as separate polymers when the NaOH fraction and its subfraction NaOH-30 were subjected to gel filtration (Fig. 1). A broad peak that appeared at the higher molecular weight region was attributed to arabinoxylans because it disappeared after digestion of this fraction with xylanase. β-Glucans were therefore responsible for the material eluted in the lower molecular weight region.

Both polymers exhibited more unusual molecular characteristics than had been previously reported for barley polysaccharides. The degree of substitution in arabinoxylans in the NaOH fraction was unusually low as indicated by a low ratio of arabinose to xylose and a high ratio of unsubstituted to substituted xylose residues (Table 1). These characteristics were more pronounced in NaOH-30 subfraction. In addition, no doubly substituted xylose residues were detected in NaOH-30. These results point to the possible presence of consecutive long runs of unsubstituted xylose residues in these polymers. β-Glucans in fractions NaOH and NaOH-30 exhibited a very high ratio of β-(1 → 4)/(1 → 3) linkages, which might indicate the presence of long blocks of contiguous β-(1 → 4) linkages, i.e., cellulose-like fragments in β-glucan chains (Table 1). These anomalous structural features possessed by both polymers

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Table 1
Monosaccharide content and linkage composition (mol%) of sodium hydroxide-extracted fractions

	NaOH	NaOH-30[a]	$NaOH_{ppt}$[b]	$NaOH\text{-}30_{ppt}$[b]
Monosaccharides[c]				
Ara	14.2	3.4	2.8	2.0
Xyl	44.0	18.7	44.2	44.8
Glc	40.9			
Ara/xyl ratio	0.32	0.18	0.04	0.04
Linkage composition[d]				
β-(1 → 4)/β-(1 → 3) ratio	5.1	6.6	12.5	13.8
Unsubstituted/substituted xylose[e]	2.6	5.3	4.2	5.8
Doubly/singly substituted xylose[f]	0.2	–	0.1	–

[a] Subfraction of NaOH fraction obtained by precipitation with 30% saturated ammonium sulphate.

[b] Precipitates obtained after digestion of fractions NaOH and NaOH-30 with lichenase.

[c] Monosaccharides were determined (in duplicate) by HPLC after hydrolysis with 1 M H_2SO_4 for 2 h at 100°C and neutralization with barium hydroxide (Izydorczyk et al., 1998a).

[d] Linkage composition was determined by methylation analysis combined with GC–MS (Izydorczyk et al., 1998a).

[e] Ratio of unsubstituted [→ 4(Xyl)1 →] to the sum of singly and doubly substituted xylose residues [→ 3, 4(Xyl)1 → + → 2, 4(Xyl)1 → + → 2, 3, 4(Xyl)1 →].

[f] Ratio of doubly [→ 2, 3, 4(Xyl)1 →] to singly substituted xylose residues [→ 3, 4(Xyl)1 → + → 2, 4(Xyl)1 →].

might permit some intermolecular alignment and/or non-covalent interactions between their chains.

More evidence of the affinity between arabinoxylans and β-glucans was obtained from experiments where the fractions NaOH and NaOH-30 were treated with the β-glucan-hydrolyzing enzyme, lichenase. Lichenase breaks specifically β-(1 → 4) linkages of glucose residues which are linked at C–O-3, and the resulting oligosaccharides are mainly tri- and tetrasaccharides (3-*O*-β-D-cellobiosyl-D-glucose and 3-*O*-β-D-cellotriosyl-D-glucose). It is also known that, upon digestion of β-glucan with lichenase, some water-insoluble material may be formed (Wood, Weisz & Blackwell, 1994; Izydorczyk et al., 1998a). This material is composed of β-(1 → 4) linked oligosaccharides with DP 9–20 and originates from the cellulose-like fragments in β-glucan chains. When fractions NaOH and NaOH-30 were treated with lichenase, large amounts of insoluble precipitate were generated. These results were expected since the linkage analysis had already indicated a high proportion of β-(1 → 4) linkages in β-glucan constituents of these fractions. However, the monosaccharide analysis of the precipitate indicated the presence, not only of glucose, but also of xylose and small amounts of arabinose. Methylation analysis confirmed the presence of unsubstituted xylose residues [→ 4(Xyl*p*)1 →] and very small amounts of singly substituted xylose residues [→ 3, 4(Xyl*p*)1 → or → 2, 4(Xylp)1 →] as well as terminal arabinose residues [(Ara*f*)1 →] (Table 1). The presence of arabinoxylans in addition to cellulose fragments in the precipitate generated by digestion of fraction NaOH-30 with lichenase was also confirmed by ^{13}C NMR analysis. The spectrum of the intact NaOH-30 fraction (Fig. 2a) exhibits resonances which indicate the presence of both polymers, arabinoxylans and β-glucans. According to the data published by Hoffmann, Roza, Maat, Kamerling & Vliegenthart (1991), the resonances in the region of 108–110 ppm are attributed to anomeric carbons of α-L-Ara*f* residues. The low intensity of these resonances in the spectrum of NaOH-30 demonstrates the low degree of substitution in arabinoxylans in this fraction, already revealed by the monosaccharide and linkage composition analyses. The resonances in the region of 100–104 ppm are attributed to anomeric carbons of β-D-Xyl*p* and β-D-Glc*p* (Dais & Perlin, 1982; Bock & Duus, 1991). The most downfield resonance at 103.9 ppm is due to C-1 of 4-*O*-substituted Glc*p* residues engaged in the (1 → 3) linkages, whereas the stronger resonance at 102.5 ppm might be because of 3-*O*- and 4-*O*-substituted Glc*p* residues engaged in the (1 → 4) linkages. The presence of the long runs of β-(1 → 4) linkages in NaOH-30 is also manifested by the relatively high intensity

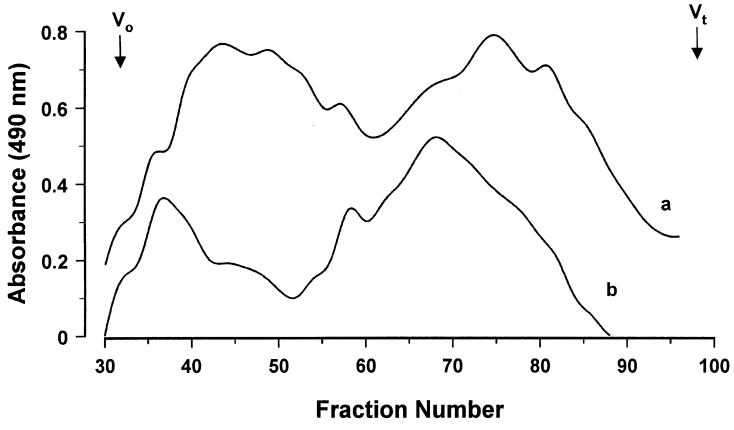

Fig. 1. Chromatography on Sepharose CL-2B column (2.5 × 95 cm^2, 0.3% NaCl and 0.05% NaN_3, flow rate 25 ml/h, 25°C) of: (a) NaOH; (b) and NaOH-30 fractions. V_0 and V_t indicate the void and total volume, respectively.

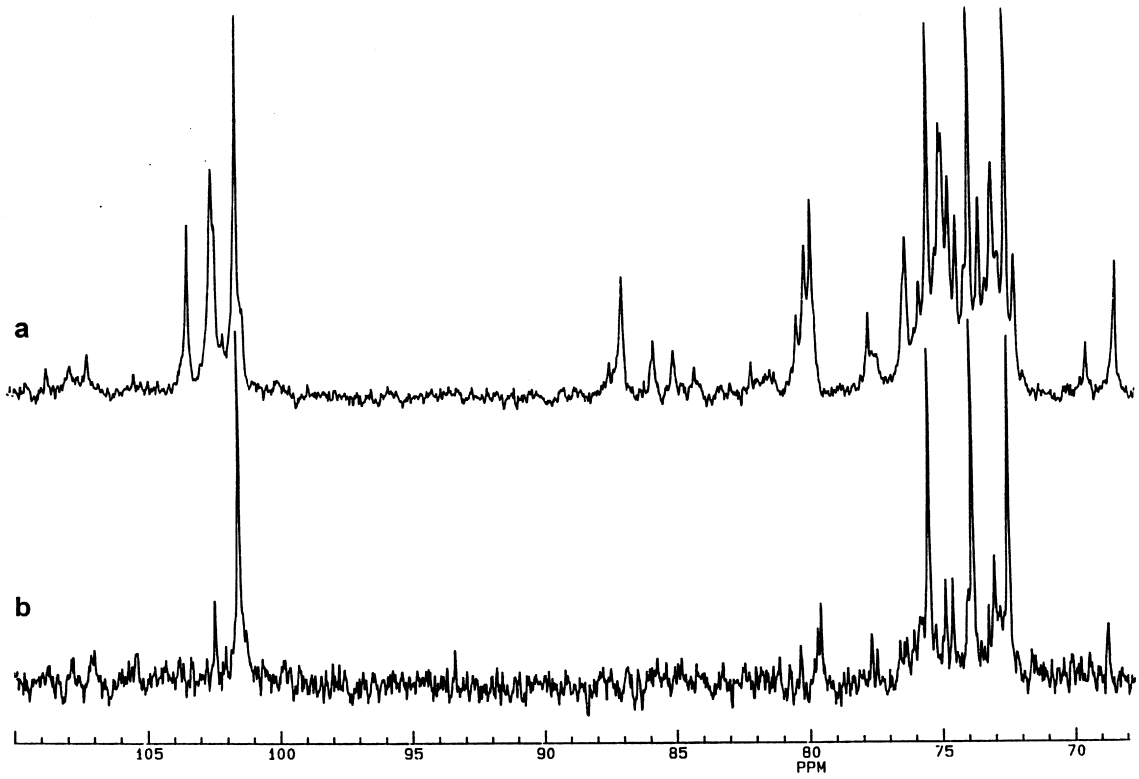

Fig. 2. ^{13}C-nuclear magnetic resonance spectra of the NaOH-30 fraction (a); and precipitate obtained after digestion of this fraction with lichenase (b). Spectra were obtained at 75 MHz on a Bruker AM 300 FT spectrometer operated at 90°C. Samples were dissolved in deuterated dimethylsulfoxide.

of the resonance at 79.4 ppm, attributed to C-4 of β-(1 → 4)-linked glucose adjoined on both sides by further β-(1 → 4)-linked Glcp residues. The intensity of the two downfield resonances at 79.6 and 79.7 ppm, assigned to C-4 of Glcp residues flanked on either the reducing or nonreducing end by β-(1 → 3) linkage, was much lower than that of the resonance at 79.4 ppm. The shoulder on the 102.5 ppm resonance might originate from C-1 of mono-substituted xylose units. The 101.7 ppm resonance has been assigned to anomeric carbons of unsubstituted xylose residues. The strong signal intensity of this resonance indicates a high content of this residue, whereas the absence of a resonance at 100.7 ppm confirms the absence of doubly substituted xylose residues in the fraction NaOH-30. The ^{13}C-NMR spectrum of the precipitate from fraction NaOH-30 (obtained after dissolving the precipitated material in DMSO) (Fig. 2b) confirms the presence of cellulose-like fragments originating from β-glucans (resonances at 102.5 and 79.4 ppm) as well as the presence of arabinoxylans (resonances at 108–110 and 101.7 ppm). The lower intensity of the resonances attributed to β-glucan oligosaccharides, compared with the stronger arabinoxylan resonances, might be due to the higher concentration of arabinoxylans in the sample analyzed (precipitated arabinoxylans probably have a greater solubility than the precipitated cellulose fragments). The presence of arabinoxylans in the precipitate generated by hydrolysis of β-glucans provides direct evidence of spontaneous and strong intermolecular association between unsubstituted regions of xylan chains and the released cellulose-like fragments from the β-glucan chains.

Even though the conformations of pure β-(1 → 4)-linked xylans and β-(1 → 4)-linked glucans are quite different (semicrystalline xylans assume three-fold left handed helices, whereas crystalline cellulose forms two-fold helices), it is possible to envisage a mechanism of interaction between β-glucans and arabinoxylans. In β-glucans, the presence of (1 → 3) linkages as well as the relatively short length of the cellulose-like fragments (compared with the chain length of cellulose polymer) would probably have an effect on their conformation. There might be too few interchain H-bonds to firmly hold to the two-fold conformation and some twisting and departure from the 'ideal' conformation might occur. Also, the xylan backbone in arabinoxylans is not expected to assume the same conformation as pure xylans. Thus, molecular interactions between arabinoxylans and β-glucans might occur, provided the length of uninterrupted β-(1 → 4) glucan and unsubstituted β-(1 → 4) xylan fragments in both polymers is sufficient to support numerous H-bonds between the chains. In the plant cell wall material, the non-covalent topological associations between β-glucans and arabinoxylans might contribute to the poor water-extractability and enzymic-indigestibility of these polymers. In solution, the

interactions of intact β-glucans and arabinoxylans are possible but probably hindered by restricted contact of the appropriate segments due to the stiff conformation of these polymers as well as interferences caused by structural irregularities (side groups in arabinoxylans and β-(1 → 3) linkages in β-glucans). On the contrary, under the conditions of hampered solvent conditions, as in the presence of ammonium sulphate, the polymer–polymer interactions are enhanced. Also, degradation of β-glucans with lichenase, which substantially increases flexibility and diffusion of chains in solution, might also facilitate better contact between the liberated cellulose-like fragments and the unsubstituted xylan blocks in arabinoxylan chains. If the interactions are numerous, aggregation and/or subsequent precipitation might occur. The form of the final product, aggregate or precipitate will probably depend on the concentration of both polymers in solution as well as on the characteristics of the solvent.

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