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Physicochemical properties and structural characterization by two-dimensional NMR spectroscopy of wheat β-D-glucan—comparison with other cereal β-D-glucans

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Abstract

The first isolation, purification and characterization of a wheat β-D-glucan is reported. Alkaline extraction of wheat bran prepared by a novel pre-processing gave a non-starch polysaccharide fraction. Purification was carried out by hydrolyzing the arabinoxylans of this fraction with β-D-xylanase (*T. Viride*, EC 3.2.1.8), which had no effect on the molecular weight of the β-D-glucan. The structure of the purified wheat β-D-glucan was characterized by NMR spectroscopy, and additional structural detail was revealed by hydrolysis with (1 → 3)(1 → 4)-β-D-glucan-4-glucanohydrolase (lichenase, EC 3.2.1.73) and analysis of the oligosaccharide reaction products. Direct and long-range homo- ($^{1}H/^{1}H$) and hetero- ($^{13}C/^{1}H$) nuclear shift correlations were used to make complete assignments of both the ^{13}C and ^{1}H spectra as well as to confirm sequences and linkage sites. The ^{13}C NMR spectrum of wheat β-D-glucan appeared identical to that of other cereal β-D-glucans, such as those from oat and barley. However, the ratio of 3-*O*-β-cellobiosyl- and 3-*O*-β-cellotriosyl-D-glucose obtained from wheat β-D-glucan by lichenase hydrolysis was significantly higher than ratios from other cereal β-D-glucans (approximately 4, 3 and 2 for wheat, barley and oat, respectively). The predominant molar proportion of trisaccharide (72.4%) from wheat β-D-glucan suggests that it will have a more regular structure than the other β-D-glucans, which might be responsible for its greater gelling ability and poorer solubility in water compared to other cereal β-D-glucans.

Keywords: Wheat β-D-glucan; NMR spectroscopy; Molecular weight (Mw); Structure

1. Introduction

Recent interest in cereal β-D-glucans has arisen largely from the potential health benefits that have led to an allowed health claim for oat products in the USA (Wood, 1997). In clinical studies, oat β-D-glucan was shown to reduce serum cholesterol levels and attenuate postprandial blood glucose and insulin response in a viscosity related fashion (Braaten et al., 1994; Wood, Braaten, Scott, Riedel & Wolynetz, 1994a; Wood, 1997). The structure, molecular weight and physical properties of oat β-D-glucan have been extensively examined (Autio, 1988; Autio, Myllymaki, Surotti, Saastamoinen & Poutanen, 1992; Beer, Wood & Weisz, 1997a; Beer, Wood, Weisz & Fillion, 1997b; Doublier & Wood, 1995; Vårum & Smidsrød, 1988; Wood, Weisz, & Blackwell, 1994b;). Barley β-D-glucan has also been extensively studied because of its significance in the malting and brewing industry (Bamforth, 1982, 1985; Gomez, Navarro, Marnzanares, Horta & Carbonell, 1997a,b; Izydorczyk, Macri & MacGregor, 1998a,b; Staudte, Woodward, Fincher & Stone, 1983; Woodward, Fincher & Stone, 1983; Woodward, Phillips & Fincher, 1988). However, little attention has been given to wheat β-D-glucan, perhaps because of its relatively low concentration in whole-wheat and refined flour (Bacic & Stone, 1980). Recently developed wheat pre-processing technology produced a novel bran fraction, in which β-D-glucan content was increased from 0.5% in the whole wheat to 2.6% in the bran fraction; in some varieties, the β-D-glucan content of the bran fractions was as high as 3% (Cui & Wood, unpublished data; Cui, Wood, Weisz & Beer, 1999; Dexter & Wood, 1996). Recently, we extracted from the pre-processed wheat bran fraction non-starch polysaccharides (NSP) that exhibited thermally reversible gelling properties (Cui, Wood, Weisz & Mullin, 1998; Cui et al., 1999). Preliminary examination showed that β-D-glucan was the responsible component for the gelling properties that are novel for wheat NSP (Cui &

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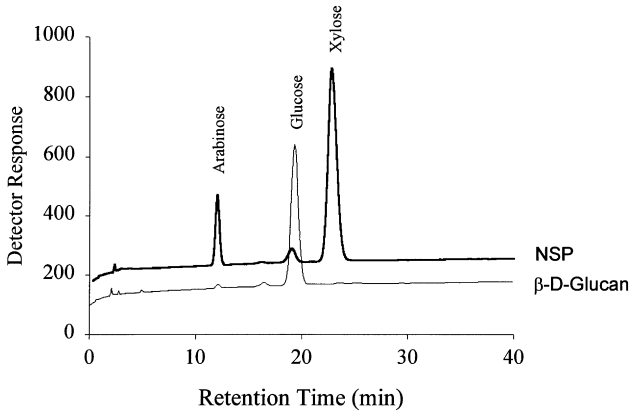

Fig. 1. Chromatographic profiles of hydrolysates from non-starch polysaccharide (NSP) and β-D-glucan extracted from pre-processed wheat bran.

Wood, 1999). This paper is a continuation of previous studies aimed at: (i) isolating and purifying β-D-glucan from the novel bran fraction; (ii) determining its fine structure by ^{13}C, ^{1}H and two-dimensional (2D) NMR spectroscopy and by specific enzyme hydrolysis; (iii) characterizing molecular weight distribution by high-performance size-exclusion chromatography; and (iv) comparing some physical properties of wheat β-D-glucan with those isolated from oat and barley.

2. Materials and methods

2.1. Materials

Barley β-D-glucan, β-D-xylanase (*T. Viride*, EC 3.2.1.8) and lichenase (EC 3.2.1.73) were purchased from Megazyme International Ireland Ltd. (Bray, Ireland). Oat β-D-glucan was prepared as described previously (Wood, Weisz, Fedec & Burrows, 1989). Pre-processed wheat bran fraction B was obtained as described earlier (Cui et al., 1998, 1999). All chemicals were of reagent grade unless otherwise specified.

2.2. Extraction and purification of wheat β-D-glucan

NSP, a mixture of arabinoxylans and β-D-glucans, was extracted from wheat bran fraction B with 1.0 M NaOH at 25°C for 2 h (solid to liquid ratio = 1 : 10) (Cui et al. 1998, 1999). The alkaline extract was neutralized with 2 M HCl and centrifuged for 20 min at 5000*g* and 25°C. The supernatant was adjusted to pH 4.75 with 0.25 M sodium acetate buffer and treated with xylanase (~100 unit/100 ml of extract) at 50°C under constant stirring for 2 h. The enzyme was deactivated by heating at 80°C for 30 min; and the solution was centrifuged for 20 min at 5000*g* and 25°C. The supernatant solution was made to 50% ethanol (final concentration), and the precipitate recovered by centrifugation. The resulting precipitate was washed with 50% ethanol, then suspended in 100% 2-propanol and kept at 4°C overnight. After removal of solvent, the precipitate was dried with gentle warming (Wood et al., 1989).

2.3. Molecular weight determination

The molecular weight distribution of cereal β-D-glucans was determined by high-performance size-exclusion chromatography (HPSEC) (Beer et al., 1997a,b) using two columns in series (Shodex Ohpak KB-806M, Showa Denko K.K., Tokyo, Japan; Ultrahydrogel linear, Waters, Milford, USA). Samples were filtered (0.45 μm) before injection of 100 μl. The columns were eluted with 0.1 M $NaNO_3$ (0.6 ml/min) at 40°C, with post column calcoflour detection (Perkin–Elmer LS-5 Spectrofluorimeter), and peak molecular weight (MWp) obtained using a calibration from 7 β-D-glucan standards (Beer et al., 1997a,b).

2.4. High-performance anion-exchange chromatography and lichenase hydrolysis

Monosaccharide composition of wheat β-D-glucan was determined by hydrolysis with 1 M H_2SO_4 at 100°C for 2 h. The hydrolysates were filtered, diluted as appropriate, and analyzed by a Dionex system (Dionex, Sunnyvale, CA) using a Carbopac PA1 column (4 × 250 mm) and guard (3 × 25 mm). Detection was by pulsed amperometry with a gold electrode.

The oligosaccharides released by lichenase were analyzed by the Dionex system using the same column as for monosaccharides but with a different elution buffer sequence. Relative amounts of oligosaccharides are calculated without correction for possible different response factors (Wood et al., 1994b).

2.5. ^{1}H, ^{13}C and 2D NMR spectroscopy

High-resolution ^{1}H and ^{13}C NMR spectra were recorded in dimethyl sulfoxide (DMSO-d6) at 500.13 and 125.78 MHz, respectively, on a Bruker AM500 NMR spectrometer operating at 90°C. A 5 mm inverse geometry $^{1}H/^{13}C/^{15}N$ probe was used. The chemical shifts were referenced to DMSO-d6 at 2.49 ppm for ^{1}H and 39.5 ppm for ^{13}C and are reported relative to TMS. Homonuclear $^{1}H/^{1}H$ correlation spectroscopy (COSY, TOCSY), and heteronuclear $^{13}C/^{1}H$ correlation experiments (HETCOR, HMBC) were run using the standard Bruker pulse sequence.

3. Results and discussions

3.1. Extraction and purification of wheat β-D-glucans

The original wheat bran fraction B contained 2.6% β-D-glucan. This β-D-glucan was not extractable by cold or hot water, possibly because of physical entanglement in phenolic cross-linked pentosans (Faulds & Williamson, 1995; Fry, 1982). 1.0 M NaOH was chosen for the extraction of β-D-glucan and arabinoxylan from residue **B** (Cui et al., 1998,

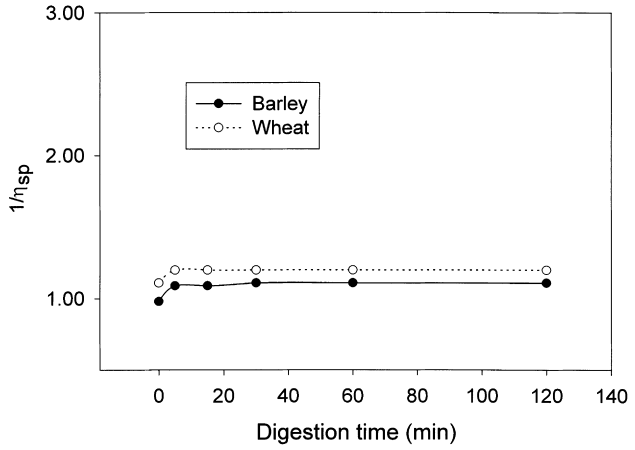

Fig. 2. Effect of β-D-xylanase on the specific viscosity (η_{sp}) of cereal β-D-glucans $\eta_{sp} = (t - t_s)/t_s$.

1999); previous data showed that the molecular weight of β-D-glucan was not affected by 1.0 M NaOH at 25°C and that a higher yield was obtained at higher alkaline concentration (Cui et al., 1998, 1999). The arabinoxylan component of the NSP was broken down to oligosaccharides by xylanase treatment; the β-D-glucan remained intact, and after precipitation by 50% ethanol was isolated essentially free of arabinose and xylose (Fig. 1).

Some batches of xylanase degraded wheat β-D-glucan as determined viscometrically, but the batch of xylanase used in this study was without action on the wheat β-D-glucan or a commercial barley β-D-glucan standard, with d/dt ($1/\eta_{sp}$) essentially zero (Fig. 2). The traditional ammonium sulfate precipitation method (Preece & Hobkirk, 1953) used in the purification of barley and oat β-D-glucans was not successful in the purification of wheat β-D-glucan. The fact that the β-D-glucan only accounted for 23% of the

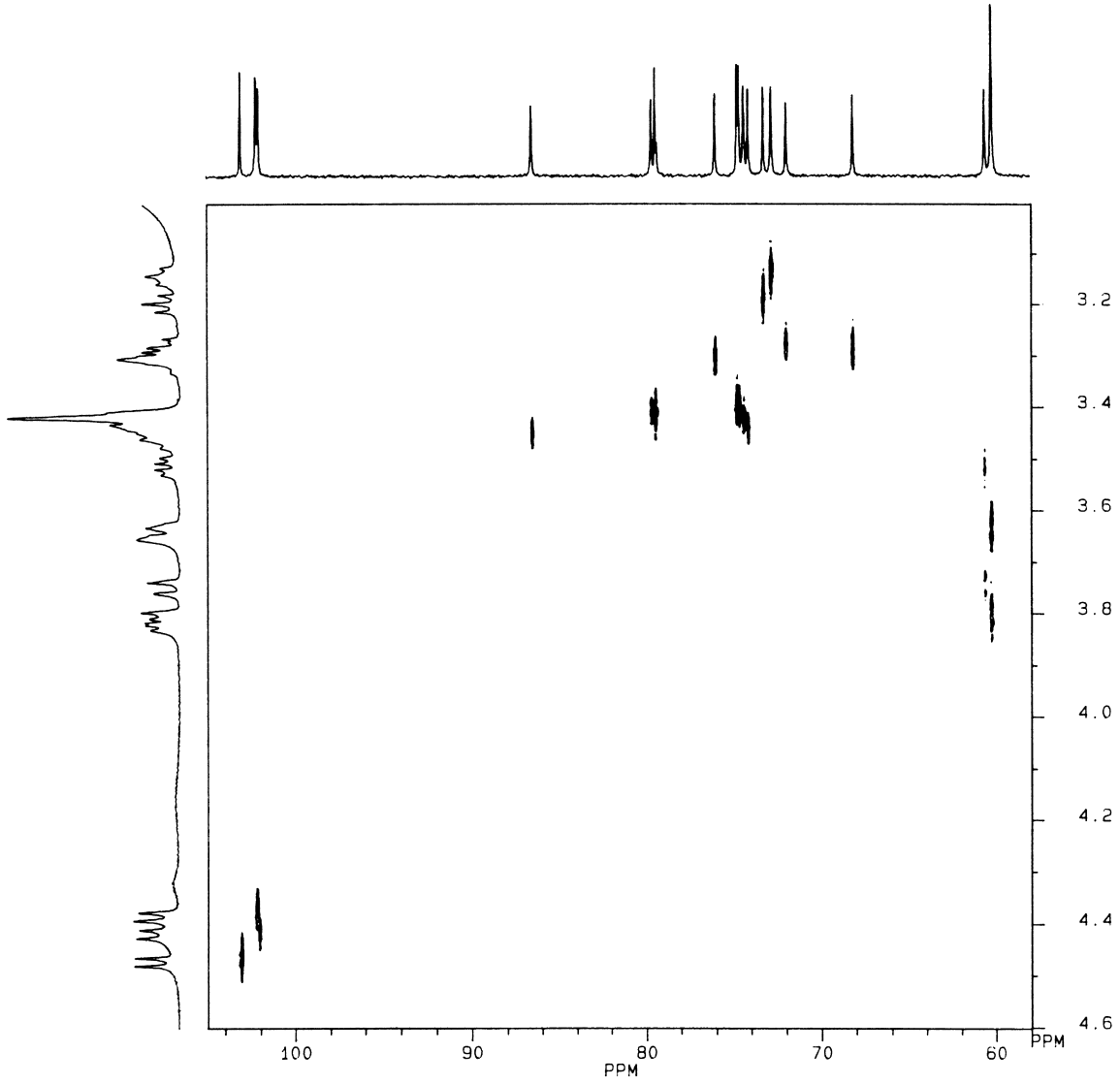

Fig. 3. ^{13}C and ^{1}H heteronuclear correlation NMR spectrum of wheat β-D-glucan in deuterated dimethyl sulfoxide.

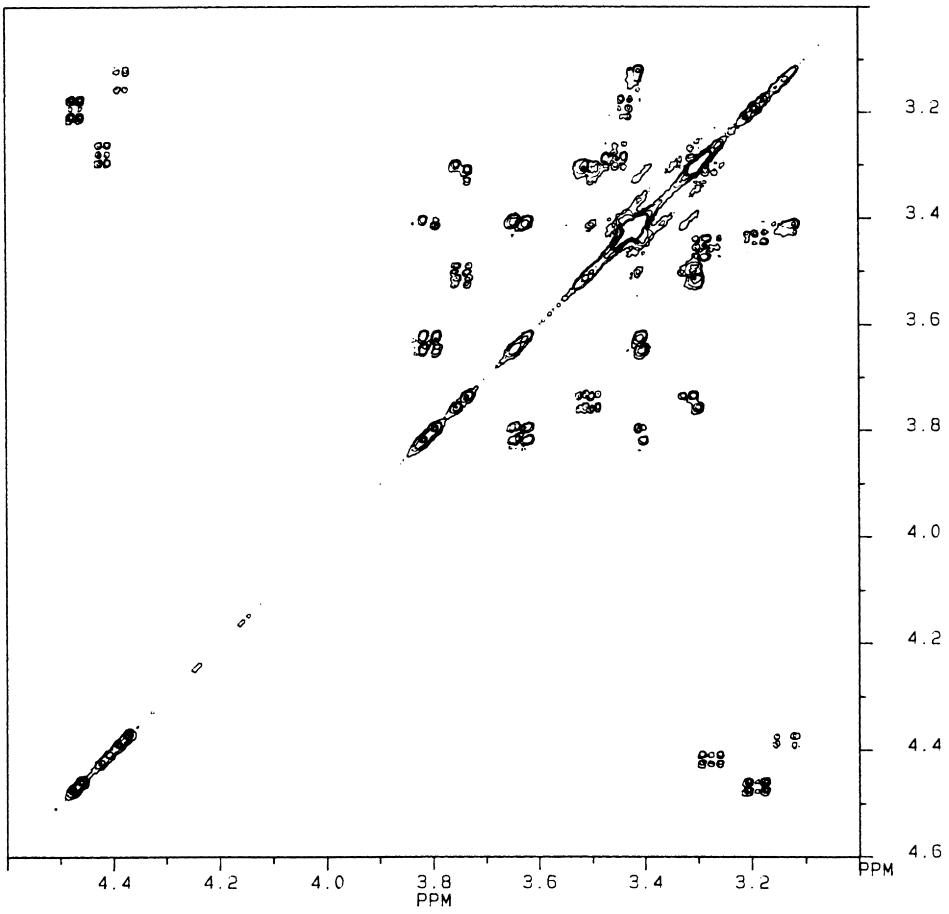

Fig. 4. Correlated spectroscopy (COSY) spectrum of wheat β-D-glucan in deuterated dimethyl sulfoxide.

total carbohydrates in the extracted NSP is a possible explanation for this phenomenon (Fig. 1).

3.2. NMR analysis

The ^{13}C NMR spectrum of wheat β-D-glucan at 90°C (projection in Fig. 3) appeared similar to that of oat and barley, as described previously (Cui & Wood, 1999). The ^{1}H spectrum, however, was well resolved compared to those of the previously studied glucans (Dais & Perlin, 1982) and showed three distinct doublets for the anomeric protons of β-D-glucan residues in three distinctly different environments (Fig. 3). $^{13}C/^{1}H$ heteronuclear correlation (Fig. 3) allowed a one-to-one match of the ^{13}C and ^{1}H resonances. The $^{1}H/^{1}H$ correlation (COSY) spectrum (Fig. 4) assigned the chemical shifts of the three H-2s from coupling with their respective anomeric protons as well as the three H-3s, which were derived from their corresponding H-2s. Similarly, the H-6 AB systems and respective H-5s were assigned, but a clear assignment of the H-4s was unobtainable due to a high degree of overlap between the H-3, 4 and 5 protons. With this knowledge of the direct correlations, a total $^{1}H/^{1}H$ correlation spectrum (TOCSY) provided all of the intra residue assignments. Sub-spectra from the 2D TOCSY spectrum corresponding to each of the anomeric resonances are shown in Fig. 5 with the single pulse spectrum (*d*) for comparison. Due to the similar values of the $^{3}J_{H,H}$ for all protons of β-D-glucose (7–9 Hz around the hexose ring), polarization is transferred completely from H-1 to H-6 in each residue, and the resolution of the anomeric protons then permits the assignment of all protons within each of the three residues. Comparison of the sub-spectra shows that one (Fig. 5b) is distinct from the others, which then might be assigned to the O-3 substituted residue (**B** in

A B C A B C D A B C

$$\rightarrow {}^{4}G_{1}\rightarrow[{}^{3}G_{1}\rightarrow {}^{4}G_{1}\rightarrow {}^{4}G_{1}\rightarrow]_{m}[{}^{3}G_{1}\rightarrow {}^{4}G_{1}\rightarrow {}^{4}G_{1}\rightarrow {}^{4}G_{1}\rightarrow]_{n}{}^{3}G_{1}\rightarrow {}^{4}G_{1}\rightarrow$$

Scheme 1.

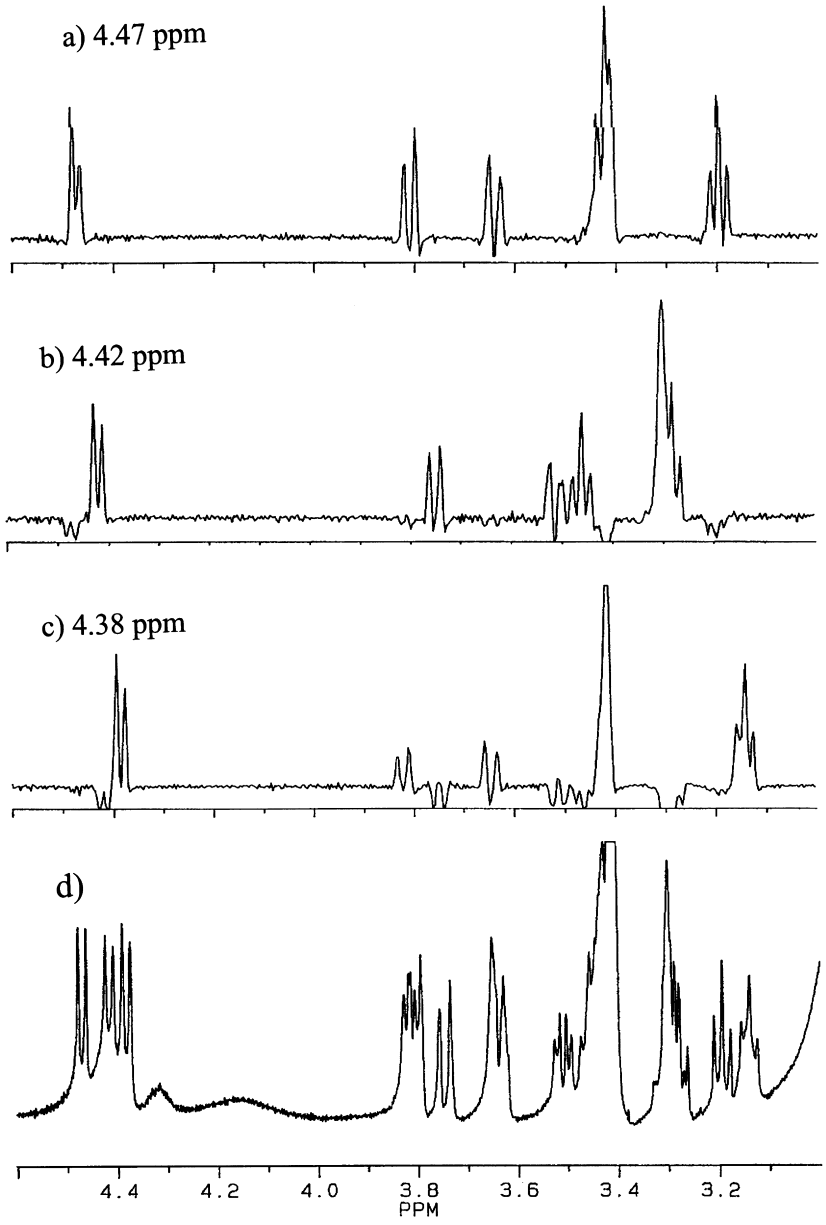

Fig. 5. Sub-spectra of the total correlation (^{1}H-TOCSY) spectrum obtained from wheat β-D-glucan in deuterated dimethyl sulfoxide. Each spectrum is a slice through the 2D spectrum at the chemical shifts of the anomeric protons, giving the intra-residue proton shifts: (a) at 4.47 ppm, corresponding to residue **A** in Scheme 1; (b) at 4.42 ppm, corresponding to residue **B**; (c) at 4.38 ppm, corresponding to residue **C**; and (d) the single pulse ^{1}H spectrum of the wheat β-D-glucan.

Scheme 1; model structure of wheat β-D-glucan), while the other two are attributed to the flanking O-4 substituted residues (**A** and **C**).

Correlations of the protons from the residue tentatively assigned to the O-3 substituted β-D-glucose residue (**B** in Scheme 1) gave carbon peaks at 101.9, 86.4, 75.9, 71.8, 68.0 and 60.5 ppm which were assigned to C-1, C-3, C-5, C-2, C-4 and C-6, respectively, shifts which confirm the (1 → 3) linkage. The presence of only a single resonance for C-3 of this residue demonstrates that, like oat and barley β-D-glucans, there are no consecutive (1 → 3)-linkages in wheat β-D-glucan (Woodward et al., 1983; Wood et al., 1994b). These assignments, along with the remaining correlations defining the two (1 → 4)-linked β-D-glucan residues, are summarized in Table 1. Overlapping resonances in the proton spectrum, however, did not permit the unambiguous assignment of C-3 and C-5s of the O-4 substituted residues, and both the carbon and proton resonances for C-6 are coincident.

Information to resolve ambiguities in ^{13}C assignments and to establish the sequence of the three β-D-glucosyl residues was obtained from analysis of the long-range heteronuclear correlation spectrum (HMBC) (Fig. 6). The important correlations arising from $^3J_{H,C,C}$ and from $^3J_{H,O,C}$

Table 1
Complete assignment of ^{13}C and ^{1}H NMR spectra of wheat β-D-glucan based on heteronuclear correlation (Fig. 3) and shift-correlated spectroscopy (COSY) (Fig. 4)

Glucosyl residue	Assigned C, H position	^{13}C resonance	^{1}H resonance
O-4 substituted (residue **A** in Scheme 1)	1	103.0	4.47
	2	73.2	3.19
	3	74.0	3.43
	4	79.3	3.42
	5	74.7	3.42
	6	60.1	3.64
			3.81
O-3 substituted (residue **B** in Scheme 1)	1	101.9	4.42
	2	71.9	3.28
	3	86.4	3.46
	4	68.0	3.30
	5	75.9	3.30
	6	60.5	3.51
			3.75
O-4 substituted (Residue **C** in Scheme 1)	1	102.1	4.38
	2	72.7	3.14
	3	74.3	3.42
	4	79.5	3.42
	5	74.6	3.42
	6	60.1	3.64
			3.82
O-4 substituted (Residue **D** in Scheme 1)	1	102.1	4.38
	2	72.7	3.14
	3	74.3	3.42
	4	79.2	3.42
	5	74.7	3.42
	6	60.1	3.64
			3.81

are summarized in Table 2. Correlations of each of the anomeric resonances at 4.47, 4.42 and 4.38 ppm to the carbon atoms across the glycosidic linkage (C-3 at 86.4 and the two C-4s at 79.5 and 79.3 ppm, respectively) define which anomeric proton can be assigned to structures **A** and **C**. Thus the anomeric proton at 4.47 is assigned to H-1 of the O-4 substituted β-D-glucosyl residue (**A**) which is glycosidically linked to the 3-position of residue **B** and attached via O-4 to another O-4 substituted residue. Similarly, the anomeric proton at 4.38 ppm is assigned to H-1 of the O-4 substituted β-D-glucosyl residue (**C**) which is linked through O-4 to C-1 of the O-3 substituted β-D-glucosyl residue (**B**), as shown in Scheme 1. The concomitant carbon to proton correlations in the reverse direction (i.e. from 103.3 ppm (C-1 of **A**) to 3.46 ppm (H-3 of **B**) and from C-1 of **B** and **C** to H-4 of **C** and **A**), are also observed; but are not as useful due to overlap of the H-4s from **A** and **C**. This, along with the additional intraresidue correlations observed between the resolved H-2s of each residue and their respective C-1 and C-3 resonances, serves to confirm the assignments within each glucosyl residue, and resolves any ambiguity between the C-4 assignments for **A** and **C**. In addition, coupling between H-3 and C-4 and H-4 and C-5 within residue **B** confirmed the relative assignments of C-4 vs C-5.

The difference between consecutive sequences of the trisaccharide and tetrasaccharide in this glucan can only be detected in the NMR spectrum through identification of residue **D**, the O-4 substituted β-D glucosyl residue which is flanked by further (1 → 4)-linked β-D-glucosyl residues. This β-D glucosyl residue behaves much like cellulosic residues, and previous studies (Dais & Perlin 1982; Wood et al., 1994b) have shown that C-4 of this residue can be assigned to the small resonance at 79.2 ppm, a shoulder on the resonance assigned to C-4 of **A**. The rest of the carbon atoms and protons for this residue are too close to those of residues **A** and **C** to be resolved. These assignments are summarized in Table 1 and are consistent with the data presented here. Comparison to the previously observed ^{13}C spectrum for the cellodextrin like residue of ~DP 9 (Wood et al., 1994b), which gives resonances at 102.1 (C-1), 79.3 (C-4), 74.7 (C-5) 74.3 (C-3) 72.8 (C-2) and 60.2 (C-1), provides an excellent match.

Although to our knowledge this is the first attempt at a complete assignment of both ^{13}C and ^{1}H spectra of cereal β-D-glucans using coupling relationships, these assignments are in complete agreement with the original assignments of Dais and Perlin (1982), especially those for residue **B**, which were based on chemical shift arguments and comparison to tetrasaccharides released by hydrolysis. These data do, however, provide a more detailed assignment of the (1 → 4)-linked β-D-glucosyl residues and sequences of the

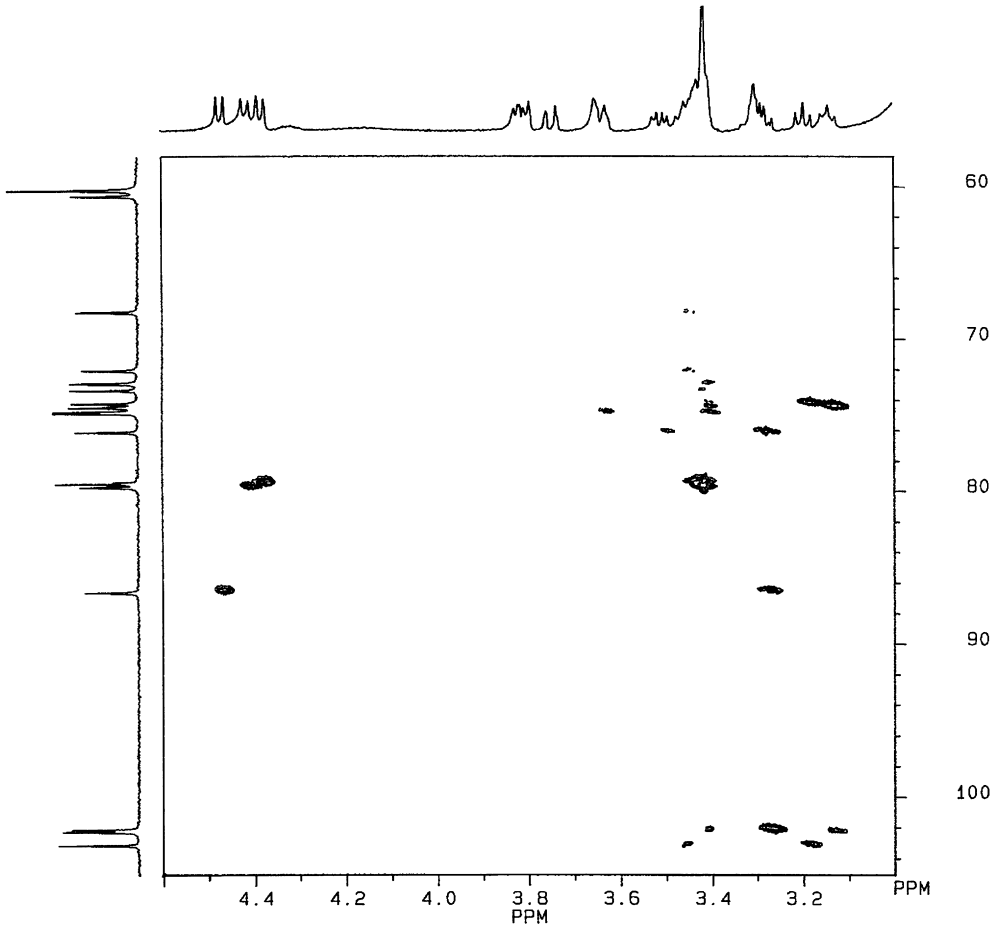

Fig. 6. ^{13}C and ^{1}H long-range heteronuclear correlation NMR spectrum (HMBC) of wheat β-D-glucan in deuterated dimethyl sulfoxide. The single pulse spectra are shown as projections.

Table 2
Linkage sequence and connectivities of wheat β-D-glucan derived from long range correlation (HMBC) spectra (Fig. 6) (**A** is the (1→4)-β-D-glucosyl residue glycosidically linked to the 3-position of the (1→3)-β-D-glucose (**B**) and attached through C-4 to a (1→4)-β-D-glucose; **B** is the (1→3)-β-D-glucose residue; **C** is the (1→4)-β-D-glucose residue which glycosidically linked to the 4-position of the (1→4)-β-D-glucose (**A** or **D**) and attached by a (1→3)-β-D-glucose at the C-4 position; **D** is the (1→4)-β-D-glucose residue which is flanked on either side by a (1→4)-β-D-glucose residues)

Resonance (ppm)	Correlated resonance (ppm)
4.47 (H-1 of **A**)	86.4 (C-3 of **B**)
4.42 (H-1 of **B**)	79.5 (C-4 of **C**)
4.38 (H-1 of **C**)	79.3 and 79.2 (C-4 of **A** and **D**, respectively)
103.3 (C-1 of **A**)	3.46 (H-3 of **B**)
101.9, 102.1 (C-1 of **B** and **C**)	3.42 (H-4 of **A** and **C**)
3.19 (H-2 of **A**)	74.0 (C-3 of **A**) and 103.0 (C-1 of **A**)
3.14 (H-2 of **C**)	74.3 (C-3 of **C**) and 102.1 (C-1 of **C**)
3.28 (H-2 of **B**)	86.4 (C-3 of **B**) and 101.9 (C-1 of B)
68.0 (C-4 of **B**)	3.46 (H-3 of **B**)
75.9 (C-5 of **B**)	3.30 (H-4 of **B**)

linkage. The resolution of the anomeric protons in the ^{1}H spectrum may also provide a method for quantitation of linkages.

3.3. Lichenase hydrolysis and oligosaccharide profile

Additional structural information was obtained by analysis of oligosaccharides released by lichenase; the relative amounts of oligosaccharide released by this enzyme constitute a fingerprint of the structure of a β-D-glucan (Wood et al., 1994a, b). As shown in Table 3, the proportion of soluble oligosaccharides of DP 3-9 from wheat β-D-glucan is significantly different from that of all other β-D-glucans (Wood et al., 1994b). The total of tri and tetrasaccharides of wheat β-D-glucan is similar to that of oat and barley β-D-glucans (92–93%); however, the molar proportion of trisaccharide from wheat β-D-glucan (72.4%) was the highest among known cereal β-D-glucans (55.0 and 62.1% for oat and barley, respectively) but lower than that from lichenan (78.1%) (Wood et al., 1994b). The ratio of tri- to tetrasaccharides of known cereal β-D-glucans follows the order wheat (4.6), barley (3.3), rye (2.7) and oat (2.2). Statistically, therefore, even though the distribution of the

Table 3
Comparison of structural features of β-D-glucans from cereals after lichenase hydrolysis

β-D-Glucan source	Peak area (%)		Total (%)		Molar ratio
	Tri[a]	Tetra[a]	Tri + tetra[b]	Penta–nona[b]	Tri/tetra[c]
Wheat bran (pre-processed)	72.3	21.0	93.3	6.7	4.5
Barley	63.7	28.5	92.2	7.8	3.3
Oat	58.3	33.5	91.9	8.1	2.2

[a] HPLC peak area percentage of trisaccharide and tetrasaccharide after lichenase hydrolysis.
[b] Total percentage of trisaccharide and tetrasaccharide and pentasaccharide to nonasaccharides, respectively.
[c] The molar ratio of trisaccharide over tetrasaccharide from lichenase hydrolysis.

β-(1 → 3)-linked cellotriosyl and cellotetraosyl units are random (Staudte et al., 1983), the likelihood of ordered repeating of the cellotriosyl units is higher for wheat β-D-glucan compared with those of oat and barley; the more ordered conformation might promote self-association. This might explain the poorer solubility of wheat β-D-glucan (freeze dried) in water compared to other cereal β-D-glucans and its greater gelling capacity (Cui & Wood, 1999).

3.4. Molecular weight distribution and physical properties

The peak molecular weight (MWp) of the chromatographic peak of wheat β-D-glucan was determined based on calibration of the HPSEC columns with seven β-D-glucan standards (Beer et al., 1997a) (Table 4). The MWp of purified wheat β-D-glucan from pre-processed wheat bran was 373 000 g/mol; the MWp of two AACC check samples and a pre-processed commercial wheat bran sample obtained from China ranged from 258 000 to 416 000. In screening of different wheat varieties, the maximum MWp for crude extracts of β-D-glucan was in the range of 500 000–800 000 g/mol. However, some batches of xylanase used to prepare isolates reduced the molecular weight to 200 000 g/mol, which indicates β-D-glucanase activity whose presence was confirmed by viscometric assay. By comparison, the MWp of crude extracts of oat and barley β-D-glucans are in the range of 1.5–2.0 million, whereas isolated oat and barley β-D-glucans are in the range of 800 000–1 500 000 g/mol (Beer et al., 1997a). The MWp of commercial barley β-D-glucan standards were 187 000 and 338 000 g/mol as determined by the same method. The apparent lower MWp of isolated wheat β-D-glucan might be the result of degradation during the extraction process. This depolymerization, if occurring, was unavoidable because the β-D-glucan was not extractable by water (even at 95°C), and the alkaline condition was necessary for solubilization. In model experiments, no depolymerization was detected using the extraction conditions described here (Cui et al., 1999). However, Beer et al. (1997a) reported that 1.25 M NaOH for 16 h at room temperature did lead to reduced MWp. There is no evidence of chemical links between the β-D-glucan and the arabinoxylan, and the insolubility of β-D-glucan may simply be due to entrapment within a matrix of phenolic cross-linked pentosans.

Table 4
Comparison of peak molecular weight (MWp) of cereal β-D-glucans

β-*d*-glucan source	MWp (g/mol)
Purified, from pre-processed wheat bran	340 000
Purified, from Chinese pre-processed wheat bran	267 000
Purified, from wheat Bran (AACC check sample, soft white)	416 000
Purified, from wheat Bran (AACC check sample, red hard)	258 000
Isolate from oat	1 160 000
Purified, from barley	708 000
Barley standard A (Megazyme)	187 000
Barley standard B (Megazyme)	338 000

Partially hydrolyzed oat β-D-glucan exhibited weak gel behavior compared to the non-hydrolyzed sample (Doublier & Wood, 1995). This suggested that the smaller molecules might have a higher mobility, which might promote self-association, possibly through the cellulose-like sequences in the structure and lead to the formation of a three-dimensional (3D) network and gel-like behavior. Similar gelling properties were observed for barley β-D-glucan standards obtained from Megazyme Intl., which might be attributed to both higher regularity in structure and relatively low-molecular weight (187 000–338 000 g/mol). Wheat β-D-glucan described here probably has a higher regularity in structure than that of barley and oat β-D-glucans. This might be related to the previous observation that wheat β-D-glucan forms gels upon cooling (Cui & Wood, 1999). Both the gelling ability and resistant to solubilisation of freeze-dried cereal β-D-glucans followed the order wheat > barley > oat; this trend corresponds to the ratio of tri- and tetrasaccharides of cereal β-D-glucans (4, 3 and 2 for wheat, barley and oat, respectively) (Cui & Wood, 1999). In separate experiments, it was also observed that a decrease of pH from 7 to 4.5 increased the solubility of wheat β-D-glucan significantly. Clear solutions are readily obtained without the extensive heating and stirring required for dissolving

β-D-glucans at neutral pH; this observation applied also to oat and barley β-D-glucans.

4. Summary

While the general structure of wheat β-D-glucan isolated and purified from pre-processed wheat bran fraction is similar to that from oat and barley, it shows difference in fine structural detail. Purification of wheat β-D-glucan was achieved by hydrolyzing the arabinoxylans of alkali-extracted non-starch polysaccharides with β-D-xylanase; however, absence of β-D-glucanase activity in this enzyme must be established. NMR spectroscopy proved to be a powerful tool in establishing the overall structure and linkage sequence of wheat β-D-glucan. Complete assignments of both ^{13}C and ^{1}H signals were achieved by direct and long-range homo and heteronuclear shift correlations. The TOCSY spectrum was used to define intraresidue proton assignments, a technique well suited for glucosyl residues due to consistency of $^{3}J_{H,H}$ around the hexose ring. The three bond $^{1}H/^{13}C$ correlation from the HMBC spectrum was used to give details of linkages and sequences. Analysis of oligosaccharides released by lichenase hydrolysis provided more detailed structural information of wheat β-D-glucan. It was found that the ratio of 3-*O*-β-cellobiosyl to 3-*O*-β-cellotriosyl D-glucose from wheat β-D-glucan was significantly higher than that from other cereals (4, 3 and 2 for wheat, barley and oat, respectively). The predominance of the repeating trimeric unit (72.4%) of wheat β-D-glucan should lead to a more regular structure, which might be responsible for its higher gelling ability and lower solubility in water compared to other cereal β-D-glucans.

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