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Evaluation of structure in the formation of gels by structurally diverse (1 → 3)(1 → 4)-β-D-glucans from four cereal and one lichen species

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Abstract

The (1 → 3)(1 → 4)-β-D-glucans from four cereal sources (oats, wheat, barley and rye) and one lichen source (Icelandic moss) were used to test two proposed structurally based hypotheses about the gelling mechanism of these polymers. Structures were evaluated using high performance anion exchange chromatography of the oligosaccharide fragments released by a (1 → 3)(1 → 4)-β-D-glucan-4-glucanohydrolase. This determined the relative amounts of cellodextrin units, of different degrees of polymerisation, which are joined by β-(1 → 3) linkages in the intact polysaccharide chain. Oat β-glucan had the lowest β-(1 → 3)-linked cellotriosyl unit content and lichenan had the highest. Strong correlations were found between the fraction of β-(1 → 3)-linked cellotriosyl units in the β-glucans and the elasticity of 6% gels in water, as measured by dynamic rheometry. Differential scanning calorimetry showed that the β-(1 → 3)-linked cellotriosyl unit content was also correlated with the onset and peak temperatures when 6% β-glucan gels were melted. No correlation was found between the longer (DP 6–9) β-(1 → 3)-linked cellodextrin oligosaccharide content and either the gel elasticity or melting characteristics. These findings are consistent with a model in which runs of consecutive β-(1 → 3)-linked cellotriosyl units form the junction zones in the gel network, but not with a model in which longer β-(1 → 3)-linked cellodextrins associate, as in cellulose fibres, to produce the gel network. Microscopic images of the β-glucan gels from the five species revealed that the microstructure was not homogeneous in any of the samples, which may be related to the variability in the enthalpy of melting of gels. There was a coarsening of gel structure as the β-(1 → 3)-linked cellotriosyl unit content increased.

Keywords: Oats; Wheat; Rye; Barley; Lichenan; β-Glucan; Gelation; Primary structure; Elasticity; Calorimetry; Microscopy; Mechanism

1. Introduction

The polysaccharide (1 → 3)(1 → 4)-β-D-glucan (β-glucan) is found in the cell walls of species of the grass family (*Graminae)* and in some lichens. The main food source of β-glucan is the endosperm cell walls of oats, but it is also present in similar amounts in barley and in lesser amounts in rye and wheat. Ingestion of β-glucan may lower postprandial blood glucose levels (Wood, Braaten, et al., 1994) and serum cholesterol levels (Braaten et al., 1994). The effectiveness of β-glucan in attenuating plasma glucose levels was diminished as the molecular weight of the polymer was reduced from 800,000 to 100,000 g/mol (Wood, Beer, & Butler, 2000). The cereal β-glucans are structurally similar to the mixed linkage β-glucan, lichenan, found in some lichen species including Icelandic moss (*Cetraria islandica*).

The primary structure of mixed linkage β-glucan is a linear chain of glucopyranosyl monomers linked by a mixture of single β-(1 → 3) linkages and consecutive β-(1 → 4) linkages. The predominant assembly is β-(1 → 3) linked cellotriosyl units which account for more than 60% of the polysaccharide (Parrish, Perlin, & Reese, 1960). In cereal β-glucans β-(1 → 3) linked cellotetraosyl units are also produced in abundance and together these two features account for more than 90% of the polysaccharide (Wood, Weisz, & Blackwell, 1991; Wood, Weisz, & Blackwell, 1994; Woodward, Fincher, & Stone, 1983). Lesser amounts of longer cellulose like regions also occur declining from 5 to 10 units or longer, but in the cereal β-glucans there is a slight increase again at eight consecutive (1 → 4)-linkages (Wood, Weisz, et al., 1994; Woodward et al., 1983). The distribution of

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the β-(1 → 3) linked cellotriosyl and cellotetraosyl units appears to be random (Staudte, Woodward, Fincher, & Stone, 1983).

Lichenan from Icelandic moss is a linear polymer of predominantly β-(1 → 3) linked cellotriosyl units. The proportion of cellotriosyl units is higher than in the cereal β-glucans (Wood, Weisz, et al., 1994). β-(1 → 3) Linked cellopentaosyl units are the second most prevalent feature, unlike the cereal β-glucans where the cellotetraosyl units are the second most common component. There are also regions of β-(1 → 3) linked cellodextrins with a higher degree of polymerisation (DP), similar to the cereal β-glucans, but there is not an increase at DP 9.

Although the focus of attention on β-glucan has been primarily viscosity, from the perspective of barley feed value (Campbell & Bedford, 1992; Jeroch & Dänicke, 1995) influence on the brewing process (Vis & Lorenz, 1997) and human nutrition (Wood, Braaten, et al., 1994), gelation behaviour was known (Letters, 1977) and exploited for isolation of lichenan (Peat, Whelan, & Roberts, 1957). Only recently have measurements of gel properties been reported (Böhm & Kulicke, 1999; Doublier & Wood, 1995) and the gelation mechanism is not fully understood. Lichenan from Icelandic moss forms gels readily in solutions of sufficient concentration. Two modes of interaction have been proposed for the nature of the junction zones in the gel network. The first involves the formation of hydrogen bonds between the longer sequences of β-(1 → 4) linked monomers into short cellulose-like fibres as shown in Fig. 1a (Doublier & Wood, 1995; Woodward et al., 1983). Alternatively, the polymers may associate through structures, which develop when several cellotriosyl units occur consecutively as shown in Fig. 1b (Böhm & Kulicke, 1999; Cui, Wood, Blackwell, & Nikiforuk, 2000). X-ray fibre diffraction studies of lichenan and barley β-glucan show that sequences of three consecutive cellotriosyl units on two polymer chains may form a stable, antiparallel complex stabilized by intermolecular hydrogen bonds between the hydroxyl groups on carbon 6 of the glucose monomers (Tvaroska, Ogawa, Deslandes, & Marchessault, 1983). The X-ray diffraction patterns suggested that while sequences of β-(1 → 3) linked cellotriosyl units are helical in contour, the intermolecular interactions are likely to be sheet-like.

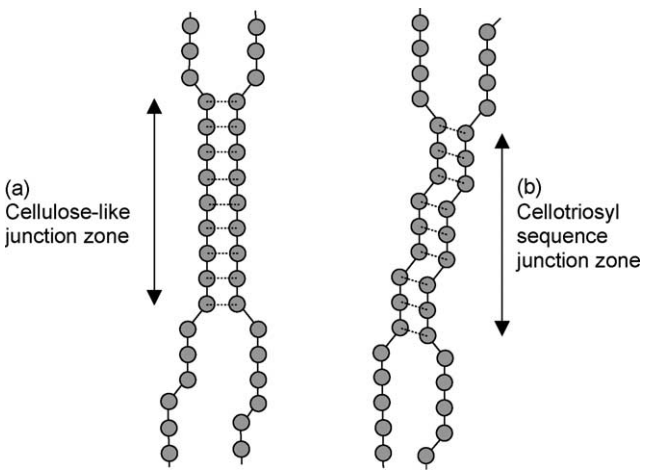

Fig. 1. Schematic diagrams of interactions that may form junction zones between (1 → 3)(1 → 4)-β-D-glucan molecules: (a) interaction between cellulose-like sections of the polymer and (b) interaction between sequential cellotriosyl units.

The gel forming properties of cereal β-glucans are strongly dependent on their molecular weight. As the molecular weight of barley β-glucans was increased from 50,000 to 300,000 mol/g the gelation time increased and the gels became less brittle (Böhm & Kulicke, 1999). For oat β-glucan in the range 35,000 to 140,000 g/mol, gelation time and gel melting temperature increased as the molecular weight increased, whereas the maximum storage modulus decreased (Lazaridou, Biliaderis, & Izydorczyk, 2003).

Hydrolysis method can also affect gelation behaviour. In a previous study, oat β-glucans were partially digested with either cellulase, which cleaves only sections of the polymer with two or more consecutive β-(1 → 4) linkages or lichenase, an enzyme which only cleaves β-(1 → 4) linkages of three substituted glucopyranosyl units. Cellulase preferentially reduces the number of cellulose-like regions, whereas lichenase is more likely to disrupt the cellotriosyl units because they are more numerous. The β-glucans hydrolyzed with cellulase produced more elastic gels than those hydrolyzed with lichenase (Tosh, Wood, Wang, & Weisz, 2004) which supports the helical structure hypothesis since the preferential degradation of the cellulose-like regions did not impair gel formation. To further test the two hypotheses, we investigated β-glucans having different primary structures and compared their gel strength and dissociation energies.

2. Theory

Mixed linkage β-glucans are linear polymers of D-glucopyranosyl units in which the linkages between the monomers can be either (1 → 4) or (1 → 3) as shown in Fig. 2. Since (1 → 3) linkages do not appear consecutively (Varum & Smidsrod, 1988) the polymers can be considered (1 → 3) linked cello-oligosaccharides of between 3 and 10 monomers. There may be trace amounts of alternating (1 → 3) and (1 → 4) linkages (Roubroeks, Mastromauro, Andersson, Christensen, & Aman, 2000) although these may only arise from end groups (Tosh, Wood, Wang, & Weisz, 2003). In Fig. 2, the degree of polymerization in the cello-oligosaccharides is designated x and the number of sequential groups of the same cello-oligosaccharide is designated n. Although oligosaccharides longer than 10 have been detected, the quantities are negligible. The literature suggests that, although the distribution of β-(1 → 3) linkages in the polysaccharide is not random, the β-(1 → 3) linked cellotriosyl and cellotetraosyl units are

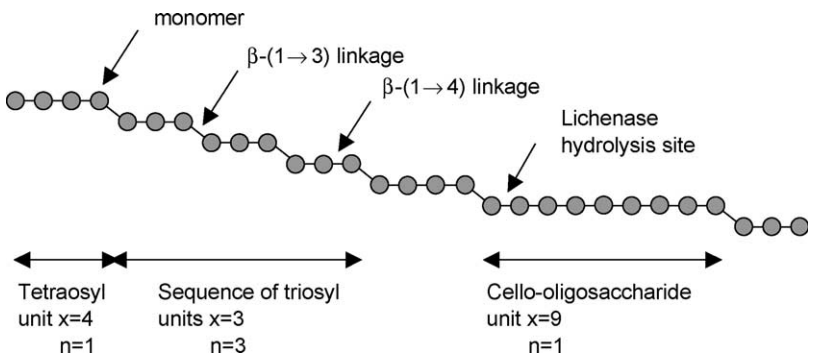

Fig. 2. Schematic diagram of a section of (1 → 3)(1 → 4)-β-D-glucan molecule defining the cello-oligosaccharide length, x, the sequence length, n, and showing a lichenase hydrolysis site. (Based on Böhm & Kulicke, 1999).

randomly distributed within the polymer chain (Buliga & Brant, 1986; Straudte et al., 1983) and we make the assumption that this is also true of the longer β-(1 → 3) linked cellodextrin units. Hence the polymer was treated as a random block copolymer and Bernoullian statistics were applied for predicting chain sequences.

To consider the option where sequential β-(1 → 3) linked cellotriosyl units form the junction zones in the gel network, we calculate the probability of finding these sequences in polymers consisting of different proportions of these cellotriosyl ($x = 3$) units.

If the probability that an oligosaccharide chosen at random is in a cellotriosyl unit is $P\{C\}$, then the probability that the next group is also a cellotriosyl unit is $P\{C\}$ (assuming that the polymer is long enough that we can ignore that one group has already been chosen) and the probability that the next oligosaccharide is a longer unit ($x > 3$) is $P\{1 - C\}$ (Ham, 1964). Thus the probability of finding a sequence of at least n consecutive cellotriosyl units is:

$$P_n\{C\} = P\{C\}^n \tag{1}$$

The probability of finding a sequence of n consecutive cellotriosyl units with a cellotetraosyl or longer unit, D, on either end is:

$$P\{DC_nD\} = \frac{P\{C\}^n P\{1 - C\}^2}{P\{C(1 - C)\}} \tag{2}$$

where $P\{C(1 - C)\}$ is equal to the total number of sequences including one or more cellotriosyl unit ($P\{DCD\} + P\{DCCD\} + P\{DCCCD\} + \cdots$) (Ham, 1964). Using Eq. (2), the frequency of sequences $x = 3$, $1 < n \leq 20$ was calculated for polymers containing 60–80% cellotriosyl units. There is an exponential decrease in the length of the sequences for any given composition but as the fraction of cellotriosyl units increases the number of longer sequences increases. By multiplying the expected number of sequences of a given length (i.e. the frequency) by the number of units in the sequence ($C{\cdot}P\{DC_nD\}$) and normalizing, the expected proportion of sequences of cellotriosyl units with increasing length is obtained (Fig. 3). Note that as the fraction of cellotriosyl units increases the maximum occurs at longer sequence lengths and the peak becomes broader.

The storage modulus is a measure of the connectivity of the gel network. G' depends on the number and strength of interaction points in the assembled microstructure. In a cured gel, the distance between junction zones is a function of frequency and proportion of reactive sites in the polymers. Because the X-ray diffraction data show that three consecutive cellotriosyl units form the stable unit cell in lichenan films (Tvaroska et al., 1983), we will consider only sequences of three or more cellotriosyl units as possible junction zones. If sequences of cellotriosyl units with $n \geq 3$ form the junction points in the gel networks of the mixed linkage β-glucan polymers, then the storage modulus of gels formed will be related to the number of reactive sites times the number of hydrogen bonds per

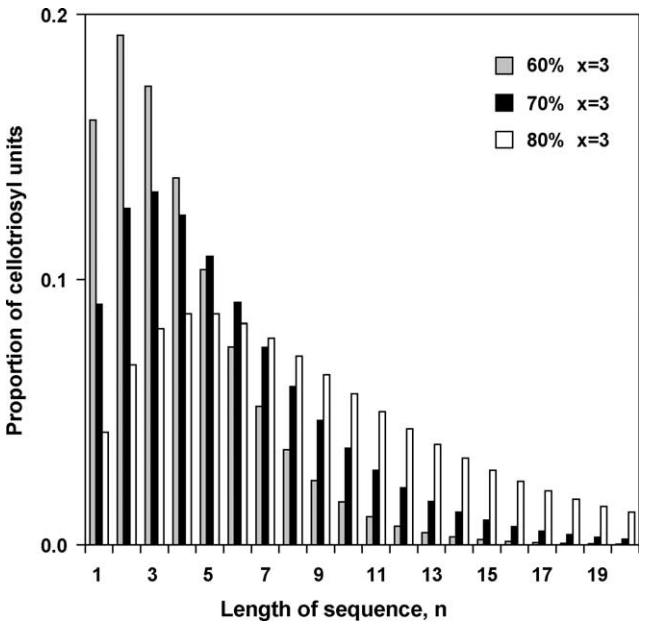

Fig. 3. The expected distribution of cellotriosyl units in sequences of different length, n, for polymers with 60, 70 or 80 molar percent of cellotriosyl ($x = 3$) units, assuming random distribution of cellotriosyl units.

junction point:

$$G' \propto \sum_{n=3}^{\infty} P_n\{C\}n \tag{3}$$

On the other hand, the shape of the endotherm and the temperature at which minimum heat flow is observed during heating, T_p, will depend on the distribution of cellotriosyl units. A peak or shoulder would be expected for each sequence of size n (for $n \geq 3$) because longer sequences would be more heat stable and T_p would occur where $P_n\{C\}$ was at a maximum.

If instead, the long cello-oligosaccharide units (cellohexaosyl and longer) form the junction zones, the number of reactive sites would be equal to the number fraction of these units, $P\{x\}$, and the storage modulus would be related to the strength of these interactions:

$$G' \propto \sum_{x=6}^{9} xP\{x\} \tag{4}$$

Again the shape of the endotherm would depend on the distribution of the longer oligosaccharides and T_p would reflect the melting temperature of the cello-oligosaccharide units with the highest $P\{x\}$.

Of course it is possible that both types of associations exist, in which case all of the reactive sites would contribute to the formation of the gel network and the value of the storage modulus. However, one mechanism is likely to be dominant over the other and only one would determine the gelling and melting temperatures.

3. Methods

3.1. Sample preparation

In order to avoid variations in gelling time and gel strength resulting from differences in molecular weight (Lazaridou et al., 2003; Tosh et al., 2004), mixed linkage β-glucans with M_w 60,000–100,000 g/mol were either purchased or prepared.

Lichenan (from *Cetraria islandica*) and barley (*Hordeum vulgare*) β-glucan samples were purchased from Megazyme International (Bray, Co. Wicklow, Ireland). The barley β-glucan sample was low viscosity (8 cSt) Lot no. 904011 and the lichenan β-glucan was Lot no. 70901.

Oat β-glucan isolate was prepared from oat (*Avena sativa*) bran by sodium carbonate extraction at pH 10, essentially as described in an earlier paper (Wood, Weisz, Fedec, & Burrows, 1989) in the POS Pilot Plant (Saskatoon, SK) except that instead of a decanting centrifuge to separate the bran from the extraction solvent, a Tolhurst basket centrifuge (Halpen Engineering, Weston, Ont.) was used. After 2.0 g oat β-glucan isolate was dissolved in 200 ml water, concentrated hydrochloric acid was added to bring the acid concentration to 0.1 N. The β-glucan was heated at 70 °C for 40 min, quickly cooled to room temperature and neutralized (pH 6.9) with 2 M NaOH. An equal volume of isopropyl alcohol was added to precipitate the β-glucan followed by centrifugation at 9700*g* for 10 min. The pellet was cut into small pieces and dewatered overnight in 100% isopropyl alcohol. The samples were dried at 80 °C and ground into powder.

Wheat non-starch polysaccharide was prepared by hot water and alkali extraction from wheat bran (*Triticum aestivum*) as described by Cui, Wood, Weisz, and Beer (1999). β-Glucan was extracted from the non-starch polysaccharide enzymatically following the method of Cui et al. (2000). Subsequently, the amylase digestion was repeated to increase the purity to 92%. The M_w of the extracted β-glucan was 388,000 g/mol and it was reduced to 63,000 g/mol by acid hydrolysis using the method described for the oat β-glucan.

Rye β-glucan was extracted from Musketeer rye (*Triticum secale*) kernels. After grinding to pass a 0.5 mm mesh, 10 g flour was refluxed in 100 ml of 70% ethanol for 3 h. The residue was isolated by washing three times with 100 ml ethanol on a sintered glass filter, dried and again ground to pass a 0.5 mm mesh. Starch was removed from the ethanol deactivated rye flour by treatment with 3000 U/ml thermostable α-amylase (Megazyme International, Bacillus lichenformis, EC 3.2.1.1). Water insoluble material (WIM) was separated from the amylase digest by centrifugation (15 min, 15,000*g*), dried and ground.

One gram of rye WIM was mixed with 100 ml saturated $Ba(OH)_2$ and stirred at room temperature for 4 h. The mixture was centrifuged (8000*g*, 15 min), the supernatant decanted and β-glucan extracted from the pellet with water (3 g WIM: 200 ml water, 75 °C for 1–2 h, pH 5–6). The suspension was cooled, centrifuged as above and polysaccharide material precipitated from the supernatant with ethanol. Precipitated material was isolated by centrifugation (12,000*g*, 15 min) and the pellet dried (3 h at 70 °C, vacuum drying 4 h at 80 °C). The extracted β-glucan was precipitated with 20% w/w ammonium sulphate. To increase sample purity, the digestion with α-amylase was repeated and the extract was then treated with xylanase (Megazyme International, source, EC 3.2.1.8), as described for wheat β-glucan (Cui et al., 2000). The purified β-glucan was precipitated by ethanol, isolated by centrifugation, dried and ground to a fine powder.

The samples were named according to their source (OG for oat gum, BG for barley gum, etc.) and weight average molecular weight, M_w. The purity of the samples was determined by the method of McCleary and Glennie-Holmes (1985) using a kit from Megazyme International.

3.2. Molecular weight determination

Size exclusion chromatography was used to determine the molecular weight, intrinsic viscosity and radius of gyration of the β-glucans. The chromatographic system was a Shimadzu SCL-10Avp (Shimadzu Scientific Instruments Inc., Columbia MD) fitted with a Shodex Ohpak Kb-806M column (Showa Denko K.K., Tokyo, Japan) and an Ultrahydrogel linear column (Waters, Milford, CT) in series. An eluent of 100 mM $NaNO_3$ with 5 mM NaN_3 at 40 °C was used at a flow rate of 0.6 ml/min. Samples (1 mg/ml) were dissolved in water at 95 °C for 3 h with stirring. The Viscotek triple detector (Viscotek, Houston, TX) had a refractive index detector, a viscometer (model 250) and a right angle laser light scattering detector (677 nm). Values were calculated using TriSEC3.0 software (Viscotek, Houston, TX) using pullulan (Shodex Std. P100, Lot no. 80301) to calibrate the instrument constants. A refractive index increment (dn/dc) of 0.146 ml/g was used.

3.3. Primary structure determination

β-Glucan (2 mg/ml) was dissolved by heating in 20 mM sodium phosphate buffer (pH 6.5) at 60 °C for 3 h with constant stirring. The solutions were cooled to 50 °C and incubated with 1 U/ml of lichenase for 90 min. The resulting oligosaccharide solutions were filtered (0.45 μm) before analysis, using high-performance anion exchange chromatography. A Carbopac PA1 column (4 × 250 mm) and guard (3 × 25 mm) were used with a pulsed amperometric gold detector (Dionex, Sunnyvale, CA). The eluents used were (A) 150 mM sodium acetate in 150 mM NaOH, and (B) 150 mM NaOH and the flow rate was 1 ml/min. The column was preconditioned with 30% A:70% B for 5 min before the oligosaccharide mixture was applied to the column. After injection, the eluent ratio was maintained at 30% A:70% B for 5 min and then a linear gradient was applied to change the ratio to 100% A over the next 8 min. The column was then washed with 100% eluent A for 12 min before preparing the column for the next sample. The pulse potentials, E, and durations, T, were $E_1 = 0.05$ V and $T_1 = 480$ ms during the measuring steps and $E_2 = 0.60$ V, $T_2 = 180$ ms, $E_3 = -0.60$ V, $T_3 = 60$ ms during the cleaning step. The response time was 1 s. The retention times were determined using glucose and oligosaccharide standards. The peak areas and retention times were calculated using Dionex software (ACI-BioLC, Sunnyvale, CA). The mole percent (mole fraction × 100) of each oligosaccharide was calculated from the relative peak areas and the molecular weights of the oligosaccharides.

3.4. Rheology

Mechanical spectra were measured on a controlled stress rheometer (CVO, Bohlin Instruments USA, NJ) fitted with a Peltier plate to control the temperature. β-Glucan (6% w/v) was dissolved by heating in 10 mM sodium phosphate buffer containing 1 mM sodium azide at 80 °C for 3 h. As the samples were dissolving, they were stirred with a magnetic stir bar and mixed with a vortex mixer at intervals. Teflon lined moulds, 1 cm in diameter, were filled to a depth of 2.8 mm. The moulds were closed tightly and stored at 5 °C for 7 days. The resultant gels were transferred to the Peltier plate of the rheometer (precooled to 5 °C) and the mechanical spectra were measured using a 1 cm flat plate (gap 2.5 mm) in control strain mode at 0.5% strain. Compression of the samples was necessary to prevent slip. Six replicates of each sample were run.

3.5. Differential scanning calorimetry

Solutions were prepared in the same manner as for the rheological measurements and held at 5 °C for 4 or 7 days. Eighty to ninety milligrams of solution or gel (4.8–5.4 mg of β-glucan) was weighed into a high volume stainless steel pan and sealed. Samples were heated from 5 to 120 °C in a Differential Scanning Calorimeter (Model 2920, TA Instruments Inc., New Castle, DE, USA) using 10 mM sodium phosphate/1 mM NaN_3 buffer in the reference pan. The onset temperature, peak temperature and enthalpy were determined using the software (Universal Analysis, TA Instruments Inc., New Castle, DE, USA) provided with the instrument. The onset temperature was the inflection point at the beginning of the peak; the peak temperature was the temperature where the heat flow was at a minimum and the enthalpy was calculated from the area of the peak. Six replicates of each sample were run.

3.6. Microscopy

Microscope slides were prepared using three cover slips and nail polish to create an enclosed space the thickness of one cover slip (0.15 mm). Two cover slips (1 cm^2) were placed on the slide about 0.8 cm apart and tacked in place with nail polish. The third cover slip was place on top of the other two to form a bridge and nail polish was used to join the cover slips together. The nail polish was then applied to the edges leaving openings at opposite corners and the slides were dried overnight. Solutions of the β-glucans were prepared in the same manner as for the rheology experiments. A slide was warmed on a glass plate heated over a water bath (80 °C). Several drops of β-glucan solution were applied to the opening on one side of the cover slip and the solution was drawn into the cavity by surface forces. The openings were sealed with nail polish and the slides were covered with plastic wrap and aged at 5 °C for 7 days. No evidence of evaporation of water from the gels (such as shrinkage or cracking) was observed after storage.

The aged gels were observed using a 63 × phase contrast, oil immersion objective lens on a Zeiss Axioskop2

Table 1
Characteristics of mixed linkage β-glucan samples used in study

Source	Sample designation	Purity % (dwb)	M_w[a] (g/mol)	M_p[b] (g/mol)	Pd[c] (M_w : M_n)	$[\eta]_w$[d] (dl/g)	Rg_w[e] (nm)
Lichenan	LN99	82	99,000	77,000	2.08	1.44	16.0
Wheat	WG63	92	63,000	41,000	1.25	1.24	13.7
Barley	BG100	100	100,000	91,000	1.30	2.12	19.0
Rye	RG63	97	63,000	51,000	1.43	1.40	14.6
Oat	OG88	92	88,000	87,000	1.53	1.73	16.8

[a] Weight average molecular weight.
[b] Peak molecular weight.
[c] Polydispersity (weight average:number average molecular weights).
[d] Weight average intrinsic viscosity.
[e] Weight average radius of gyration.

light microscope (Carl Zeiss MicroImaging, Inc., Thornwood, NY). The images were captured and the calibration mark calculated using a Spot CCD camera and Advanced Spot software from Diagnostic Instruments, Inc. (Burlingame, CA).

3.7. Statistical analysis

Significant differences between β-glucans from different sources and correlations between test parameters were determined using the data analysis package in Microsoft® Excel. Single factor analysis of variance, correlation and *t*-test analysis tools were used.

4. Results and discussion

The purity of the β-glucan isolates ranged from 82 to 100% β-glucan (Table 1). The weight average molecular weights were between 63,000 and 100,000 g/mol and the polydispersities of the preparations were similar as shown in Table 1.

The normalized proportions of the oligosaccharides released by lichenase, based on the area percentage, are shown in Table 2 along with the tri:tetra molar ratios of the β-glucans and sum of the hexa- to non-saccharides. Because pure standards of lichenase digest products, 3-*O*-β-cellobiosyl-D-glucose, 3-*O*-β-cellotriosyl-D-glucose, etc. are not available, the exact weight fraction of the oligosaccharides cannot be calculated. However, the response factor, R, decreases as the DP increases in any homologous series (Wood, Weisz, et al., 1994). Valid comparisons of like oligosaccharides, however, can be made between the structures of different species using the molar percentages, M%, calculated. For instance,

$$P\{C\} = \mathrm{M}\%R_3 \tag{5}$$

where R_3 is a constant for 3-*O*-β-cellobiosyl-D-glucose, and therefore,

$$P\{C\}_{\mathrm{barley}} - P\{C\}_{\mathrm{oat}} = R_3(\mathrm{M}\%_{\mathrm{barley}} - \mathrm{M}\%_{\mathrm{oat}}) \tag{6}$$

Similarly,

$$\sum_{x=6}^{9} P\{x\} \propto \sum_{x=6}^{9} \mathrm{M}\%_x R_x \tag{7}$$

where R_x is a constant response factor for each oligosaccharide of length x.

Lichenan has features that distinguish it from the cereal β-glucans. It has the highest 3-*O*-β-cellobiosyl-D-glucose (DP3) content and the lowest 3-*O*-β-cellotriosyl-D-glucose (DP4) content giving it a tri:tetrasaccharide ratio much higher than the cereal β-glucans. It also has a relatively high pentasaccharide level. There is evidence that the activation energy of crystallization for cello-oligosaccharides with an odd number of units, like cellotriose, is lower than those with an even number, like cellobiose and cellotetraose (Hatekeyama, Yoshida, & Nakano, 1976). This suggests that there may be a preference for such glucans to associate through odd numbered units and therefore a sufficient number of consecutive pentaosyl units may promote

Table 2
Molar percentages of the oligosaccharides derived from the five species of mixed linkage β-glucans by complete digestion with lichenase

Sample	DP3	DP4	Tri:Tetra	DP5	DP6	DP7	DP8	DP9	DP6-9
LN99	86.35	2.71	31.90	6.82	1.86	1.16	0.85	0.26	4.13
WG63	79.19	16.56	4.78	2.41	0.93	0.40	0.18	0.33	1.85
BG99	75.84	20.89	3.63	1.78	0.80	0.18	0.15	0.36	1.50
RG63	73.73	21.37	3.45	2.61	0.97	0.56	0.27	0.50	2.30
OG88	67.32	28.57	2.36	1.96	1.11	0.30	0.31	0.42	2.15

The molar ratio of DP3:DP4 and the sum of the DP6-9 are indicated. DP3, 3-*O*-β-cellobiosyl-D-glucose; DP4, 3-*O*-β-cellotriosyl-D-glucose and longer oligosaccharides follow the same pattern.

gelation more than consecutive tetraosyl units. The DP3 content of the cereal β-glucans ranged from 67.3 M% for oat β-glucan to 79.2 M% for wheat β-glucan. Since each DP3 is derived from a β-(1 → 3) linked cellotriosyl unit and the β-(1 → 3) linked cellotriosyl units are randomly distributed, the differences in sequence length should be similar to those seen in Fig. 3. We would expect a smaller proportion of the β-(1 → 3) linked cellotriosyl units in oat β-glucan to occur in sequences of three or more units and that the average sequence length would be shorter. Thus, if the β-(1 → 3) cellotriosyl sequences form the junction points in the gel network, the density of potential interaction points is lowest in oat β-glucan and highest for lichenan. Although the peak area of the oligosaccharides in the cereal β-glucans decrease sequentially from DP3 to DP8, the peak area of DP9 is greater than DP8. The sum of the longer cello-oligosaccharides (DP6-9) ranges from 1.5 M% for barley β-glucan to 4.13 M% for lichenan and the order of increasing M% in the cereal β-glucans is different from the order of increasing DP3 content. Therefore, the expected order of increasing elasticities and melting temperatures would be different for the two hypotheses.

The 6% (w/w) gels aged 7 days at 5 °C showed typical elastic gel behaviour. For low M_w oat β-glucan, a plateau in G' has been reached at 7 days (Tosh et al., 2004). The storage modulus, G', was frequency independent over the range of 0.1–10 Hz and the loss modulus, G'', had a positive slope and was more than an order of magnitude smaller. The average G' values (for 0.01–10 Hz) are shown in Fig. 4. The average G' is linearly correlated ($r^2 = 0.996$) with M% for DP3 (Fig. 5), but there is no correlation between G' and the M% DP6-9 (Fig. 6). Indeed, the oat β-glucan had the second highest DP9 content but the lowest

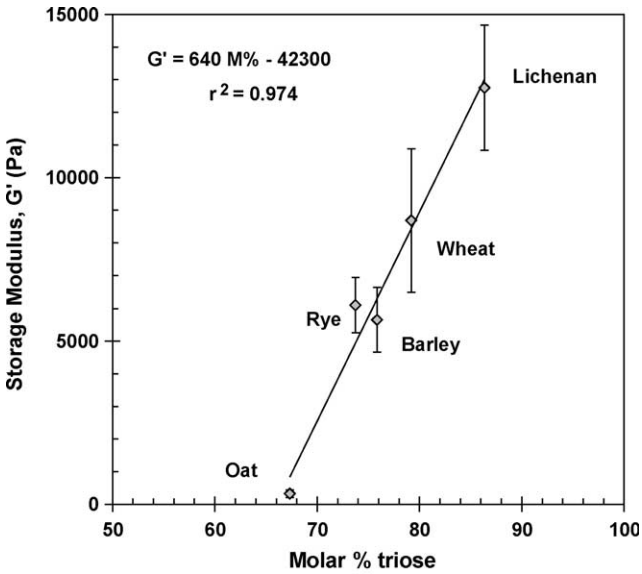

Fig. 5. Correlation of storage modulus, G', with the mole % cellotriosyl units in each of the (1 → 3)(1 → 4)-β-D-glucans. Gels were aged 7 days at 5 °C.

G' value. Since the elasticity of the gel is dependent on the number of junction points in the network, the correlation for G' with DP3 indicates that the β-(1 → 3) linked cellotriosyl units are involved in the intermolecular interactions.

Typical endotherms showing the heat flow in the five gels as they melted in the DSC are shown in Fig. 7. The endotherms are broad (30–50 °C) and show evidence of shoulders each side of the main peak. The breadth of the endotherms is indicative of polydispersity of the molecular interactions. The junction zones appear to have a large variation in the number of hydrogen bonds, as

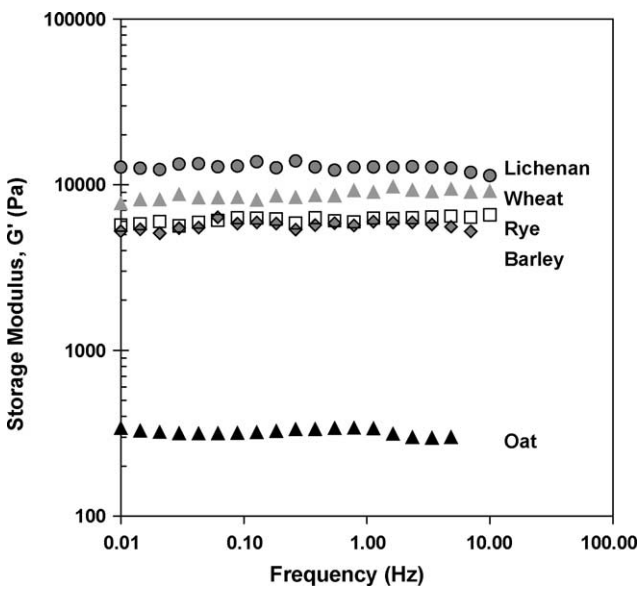

Fig. 4. Average storage moduli, G', of 6% β-glucan gels during a frequency sweep at 0.5% strain and 5 °C. Gels were aged 7 days at 5 °C.

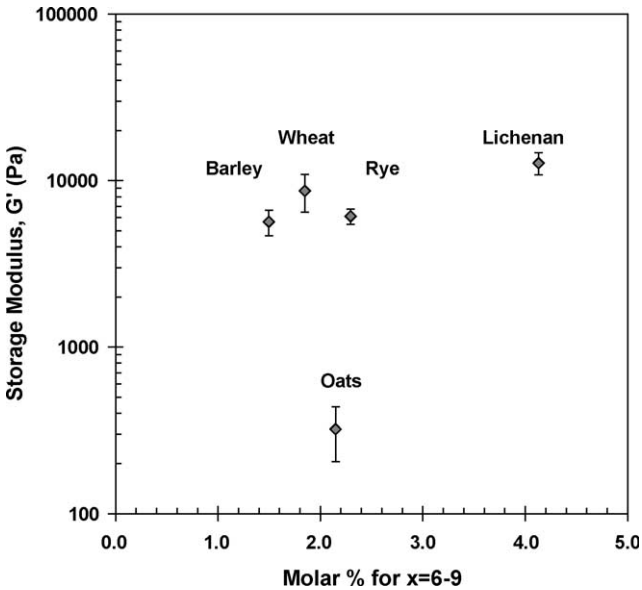

Fig. 6. No significant correlation is found between storage modulus, G', and the sum of the mole % of longer cello-oligosaccharides ($x = 6 \rightarrow 9$).

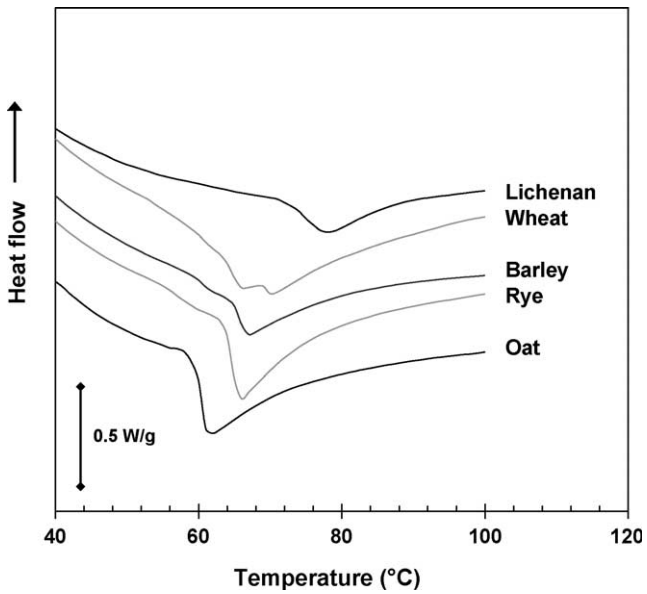

Fig. 7. Typical endotherms for melting of gels aged 7 days at 5 °C. Exotherm up.

would be expected for either mechanism. The shape of the endotherms can be related to the shape of the frequency distributions in Fig. 3 for cellotriosyl contents of 0.6–0.8. The peaks become broader and shift to higher temperatures as the cellotriosyl content increases which is consistent with the cellotriosyl sequence hypothesis. The oat β-glucan has the lowest β-(1 → 3) linked cellotriosyl unit content, which would result in shorter average length of reactive sites. The shorter sequence length junction zones would have lower melting temperatures than junction zones made up of longer sequence zones, which would be, statistically more numerous in rye, barley, wheat and lichenan β-glucans, in ascending order. Shoulders on the peaks would correspond to junction zones that had fewer or more hydrogen bonds than the most frequently occurring junction size. The increase in peak width results from a larger diversity in the size and number of hydrogen bonds in the junction zones of the gel microstructure. A β-glucan containing 60% β-(1 → 3) linked cellotriosyl units would be expected to have junction zones made up primarily of $n = 3$ sequences (Fig. 3) whereas a β-glucan containing 80% β-(1 → 3) linked cellotriosyl units would have junction zones made up of a wider range on sequence lengths with the most frequent lengths being $n = 4$ and 5. Therefore, it was expected that oat β-glucan would have the narrowest endotherm and lichenan the widest endotherm.

Both the onset and peak temperatures, T_o and T_p, respectively, were linearly correlated with the percentage of β-(1 → 3) linked cellotriosyl units in the β-glucans (Fig. 8). T_o indicates the melting temperature of the least stable junction zones and increases with increasing cellotriosyl content. Thus, the interactions between

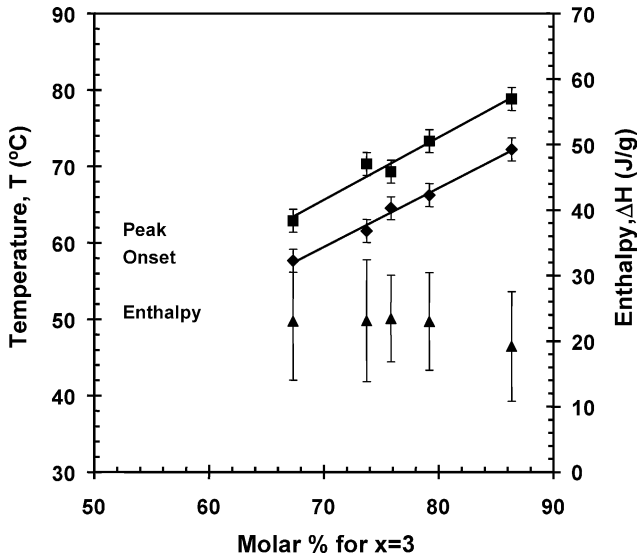

Fig. 8. Correlation of peak and onset temperatures, T_p (■) and T_o (◆), respectively, with mole % cellotriosyl units in the (1 → 3)(1 → 4)-β-D-glucans tested. $T_p = 0.82$ M% + 8.32 ($r^2 = 0.969$). $T_o = 0.77$ M% + 5.44 ($r^2 = 0.991$). No significant differences in melting enthalpy (▲) were observed among the β-glucans.

molecules of oat β-glucan, with the lowest cellotriosyl content, occur with fewer hydrogen bonds either because they involve fewer parallel strands or shorter sequences and result in the lowest T_o. The shift of the peak temperature, T_p, to higher temperatures, with increasing cellotriosyl content, substantiates the cellotriosyl junction zone hypothesis. The linear increase in T_p corresponds to the predicted shift in proportion of cellotriosyl units in longer sequences as shown in Fig. 3. As the cellotriosyl

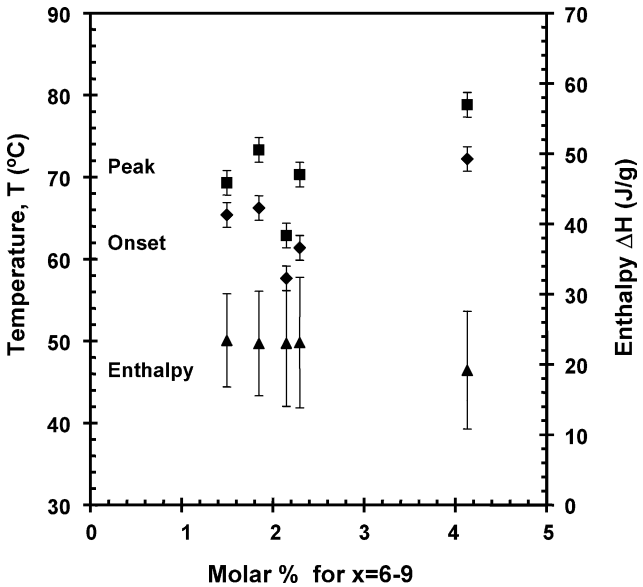

Fig. 9. Peak and onset temperatures, T_p (■) and T_o (◆), respectively, were not found to be correlated with the sum of the mole % of longer cello-oligosaccharides ($x = 6 \rightarrow 9$). Melting enthalpy (▲) was not significantly different for the β-glucans).

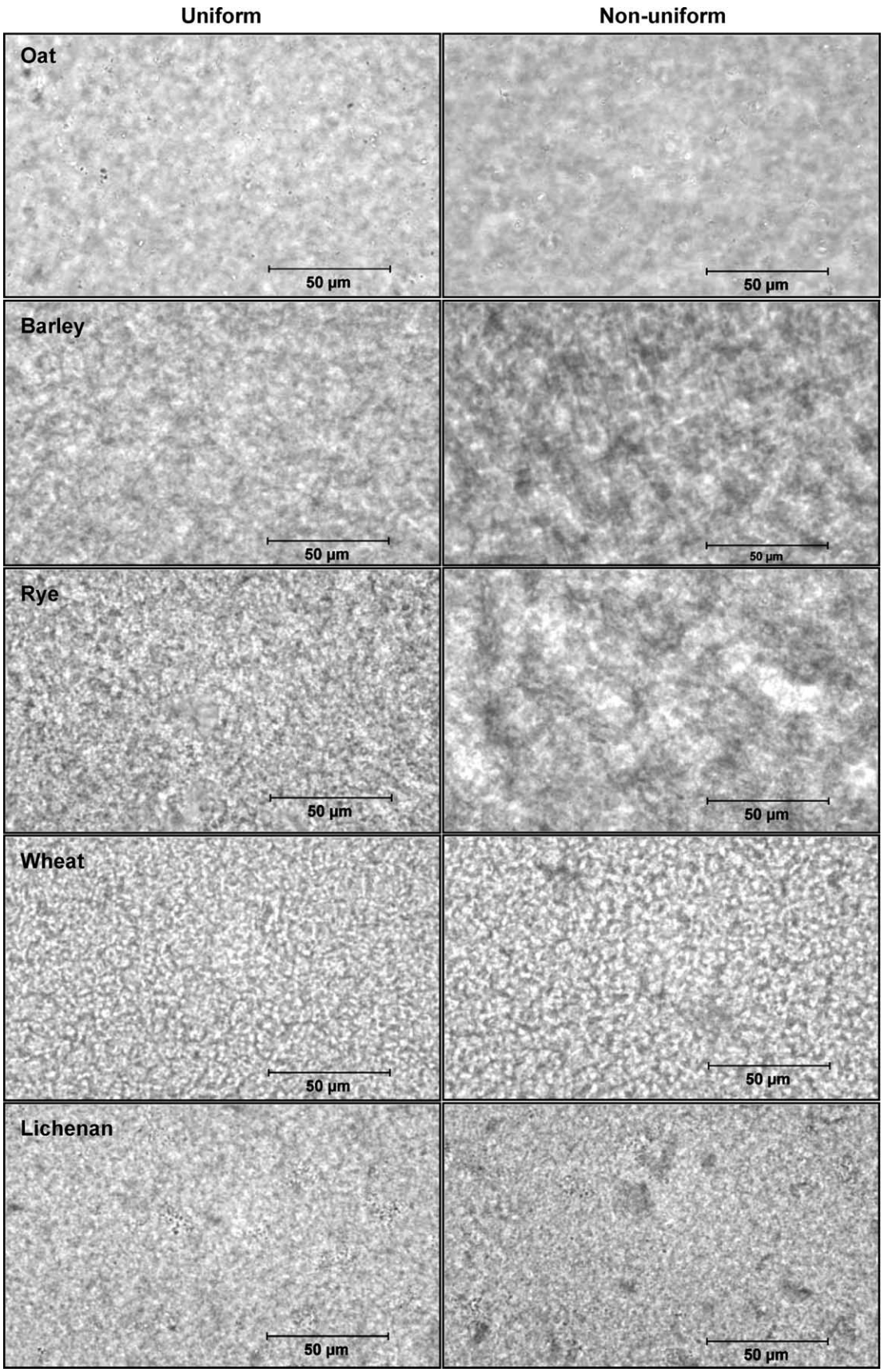

Fig. 10. Phase contrast microscopy images of 6% β-glucan gels aged 7 days at 5°C. Images in the left column show areas of uniform structure whereas images in the left column show areas in the gels where the structure is not homogeneous.

content increased, the average length of the sequences increases allowing for the formation of longer, more stable junction zones in the gel network. The slope for T_p (0.82) is greater than the slope for T_o (0.77), which reflects the broadening of the peaks which is also predicted by the cellotriosyl junction zone hypothesis and illustrated in Fig. 3. This increase in T_p reflects an increase in the average stability of the junction zones stabilizing the gel network and would be expected for longer sequences of cellotriosyl units, which occurs as the cellotriosyl content increases. Thus, the cellotriosyl sequence model (Fig. 1b) predicts an increase in T_p and a broadening of the endotherm for polymers of increasing cellotriosyl unit content.

If cellulose-like associations were the primary mode of gel development, we would expect that T_p would reflect the melting temperature of the most numerous oligomer, in this case an assembly of DP6 segments, since DP6 is the predominant oligomer $>$DP5. Therefore if the cellulosic junction zone hypothesis were correct, we would expect T_o and T_p to be constant for all the β-glucans. The significant differences among the cereal β-glucans suggest that neither DP6, nor the total compliment of DP6-9 are involved (Fig. 9). Therefore, shifts in T_o and T_p are not compatible with the cellulosic junction zone model.

Significant differences in ΔH for the five species were not observed ($p = 0.33$), though the variability within the samples would make it difficult to detect small differences between species. Differences in enthalpy could be masked by the inhomogeneous distribution of junction zones in the gel structure. Since the size of the sample used in the DSC pans was a significant portion of the gel (80 of 900 mg) it seemed unusual that there should be such large variations in enthalpy between samples taken from same gel. In order to further investigate the microstructure of the gels, gels of uniform thickness were viewed in the light microscope.

Microscopy confirmed that although the gels appeared to be translucent and homogeneous with the naked eye, the microstructure contained inhomogeneities. In Fig. 10, two fields of view from the same sample are shown, where each field of view contains approximately 3 mg of 6% β-glucan gel. The images on the left show areas where the structure was fairly uniform and the images on the right show areas where the microstructure contained nodes of dense structure. These nodes contained higher densities of polymer and would have more hydrogen bonds per unit volume. Variability in the number and size of nodes in samples may account for the large differences observed in the area of the endotherm peaks. In the homogeneous regions, the optical density of the gel appeared to increase from top to bottom which is the same order as the increase in β-(1 → 3) linked cellotriosyl units within the primary structure. This increase in density of the junction zones of the gel structure would account for the increase in peak and onset temperatures as measured by DSC. Junction zones that are more compact would have more hydrogen bonds per unit volume and would require more thermal energy to disrupt them.

In the non-homogeneous structures there appeared to be a type of superstructure where the gel had alternating areas of high and low density. The non-uniform regions in the barley and rye gels appear to have two levels of structure: short correlation lengths similar to the uniform regions and a superstructure with correlation lengths around 10 μm. The variability of the structure within the samples could account for the variability of the enthalpy in the DSC measurements. The apparently inhomogeneous distribution of β-glucan in the gels is interesting and warrants further investigation.

5. Conclusion

The behaviour of the gels formed from solutions of cereal β-glucans and lichenan are consistent with the hypothesis that sequences of consecutive cellotriosyl units in the polymer chain are involved in the mechanism through formation of stable junction zones. The elasticity of the gel, and the peak temperature in the melting endotherm, increased linearly with cellotriosyl content as predicted by Bernoullian chain statistics for this model. The evolutionarily conserved nonsaccharide feature in the polymer structure may have some biological function but it does not appear to be related to gel formation or to self-association in mixed linkage (1 → 3)(1 → 4)-β-glucans after extraction from the cell walls.

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