

# Molecular Characterization of Barley $\beta$ -Glucans by Size-Exclusion Chromatography with Multiple-Angle Laser Light Scattering and Other Detectors

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## ABSTRACT

Cereal Chem. 74(5):599–604

Molecular characteristics were determined for mixed-linkage (1 $\rightarrow$ 3) (1 $\rightarrow$ 4)- $\beta$ -D-glucans ( $\beta$ -glucans) extracted from Azhul, Crystal, Waxbar, and Prowashonupana barleys.  $\beta$ -Glucans in extracts (with or without  $\alpha$ -amylase, protease, hemicellulase, or xylanase treatment) were separated from other components by high-performance size-exclusion chromatography and detected with multiple-angle laser light scattering, refractive index, and fluorometry following postrefractive index treatment with Calcofluor. Pretreatment of barley with 70% ethanol (80°C, 4 hr) reduced

$\beta$ -glucanase activity by ~20%. Hot-alcohol treatment also reduced  $\beta$ -glucan extraction at 23 and 65°C by 42 and 14%, respectively. Molecular weights of  $\beta$ -glucans in the first water extract were generally higher than in succeeding water and alkali extracts. Weight average molecular weights ranged from  $0.44 \times 10^6$  to  $2.34 \times 10^6$  g/mol after  $\alpha$ -amylase treatment to remove interfering starch. Interference due to pentosans was not demonstrated using enzyme treatments.

Soluble dietary fiber including  $\beta$ -glucans are reported to lower plasma cholesterol and postprandial serum glucose in humans and animals (Anderson et al 1990, Anderson and Akanji 1993, Jenkins et al 1993, Tietzen 1993, Glore 1994, Hallfrisch et al 1995). Although the relationship of physical properties and physiological responses is still unclear (Eastwood 1992), high viscosity and solubility of  $\beta$ -glucans are thought to be necessary for their physiological properties (Anderson et al 1990). Physical properties of these carbohydrate polymers, reported to vary with source and variety of barley (Bhatty 1987, Xue et al 1991), are related to molecular size and shape. Milling, extraction, digestion, and other processes can degrade  $\beta$ -glucans. The ability to measure size and shape of  $\beta$ -glucan polymers in cereal foods is necessary to the elucidation of mechanisms for  $\beta$ -glucans' physiological responses. Molecular characterization of  $\beta$ -glucans have been made by separating molecules using high-performance size-exclusion chromatography (HPSEC) and determining molecular weight and shape with multiple-angle laser light scattering (MALLS) detection (Williams et al 1991a,b; Rollings 1992). The determination of molecular weight (MW) by MALLS is considered an absolute method that does not require universal calibration using standards of known molecular weight and low polydispersity. At present, suitable polysaccharide standards >800,000 MW are not available. The theory and use of MALLS for molecular characterization was reviewed (Wyatt 1993).

Extraction and sample preparation are important first steps in molecular characterization. Alcohol treatment has been used to prevent degradation by endogenous enzymes during isolation of cell-wall fractions and extraction of  $\beta$ -glucans (Forrest and Wainwright 1977; Ballance and Manners 1978; Wood et al 1983, 1991; Beer et al 1995). Enzymes have been used to isolate and characterize cell-wall components (Parrish et al 1960, Ballance and Manners 1978, Selvendran and Du Pont 1980, Dais and Perlin

1982). Degradation of  $\beta$ -glucans by salivary  $\alpha$ -amylase was either not considered or not observed when the enzyme was used with amyloglucosidase or pullulanase to remove residual starch from cell-wall isolates (Ballance and Manners 1978, Selvendran and Du Pont 1980).

In addition to analytical purposes, extraction may also be a step in producing products that are totally soluble and more suitable for substitution into a wider range of food products. Solubility differences of  $\beta$ -glucans with temperature could mean differences in their molecular weights. This article compares molecular weights of  $\beta$ -glucans extracted at different temperatures and identifies, by enzyme treatments, possible substances that interfere with the measurement of molecular characteristics of  $\beta$ -glucans using HPSEC/MALLS.

## MATERIALS AND METHODS

### Barley

Azhul (grown in Arizona), Crystal (grown in Washington), Waxbar (grown in Montana), and Prowashonupana (ConAgra, Omaha, NE) were obtained in late 1994 and stored unground under ambient conditions until assay in 1995. For analysis, barleys were ground in a Udy mill fitted with a 0.2-mm screen. A  $\beta$ -glucan-enriched barley (Waxbar) fraction (Knuckles and Chiu 1995) was also included in this study. Composition of the materials is given in Table I.

### Extractions

Samples were treated with 70% ethanol at reflux for 4 hr before extraction. The alcohol was removed by decantation and air dry-

TABLE I  
Composition of Barley Flours<sup>a</sup>

Component, %	Azhul	Crystal	Waxbar	Enriched Waxbar	Prowashonupana
Moisture	9.7	10.1	9.3	9.4	7.9
Nitrogen	1.9	1.8	1.6	2.1	3.3
Fat	3.1	2.9	2.5	3.4	6.2
Fiber (total)	20.3	13.7	13.9	42.1	30.1
Ash	2.2	1.3	2.2	3.1	2.5
$\beta$ -Glucans	5.2	5.7	5.9	18.9	14.7
Pentosans	3.7	nd <sup>b</sup>	3.0	8.5	nd

<sup>a</sup> Dry weight basis, except for moisture. Means,  $n = 2$ .

<sup>b</sup> Not determined.

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ing. Total  $\beta$ -glucans were determined by extracting samples (100 mg) with 1N NaOH containing 1% NaBH<sub>4</sub> (10 mL, 1 hr, 65°C).  $\beta$ -glucans were also sequentially extracted from the same sample (250 mg) with water (20 mL, 1 hr, 23°C), water (20 mL, 1 hr, 65°C), water (20 mL, 1 hr, 100°C), and 1N NaOH containing 1% NaBH<sub>4</sub> (10 mL, 1 hr, 65°C). NaOH-NaBH<sub>4</sub> extracts were adjusted to a pH level appropriate for enzyme treatment or chromatography (pH 11.0–11.5) with 5N acetic acid and diluted to 20 mL.

### Enzyme Treatments

Hot water and NaOH-NaBH<sub>4</sub> extracts can contain starch and pentosans (arabinoxylans) that may coelute and interfere with the MW determinations of  $\beta$ -glucans (Forrest and Wainwright 1977, Bhatti et al 1991). Enzyme treatments were used to distinguish between  $\beta$ -glucans and interfering molecular species. Aliquots of each extract were chromatographed without enzyme treatment. Other aliquots of extracts were treated with individual enzymes or in combinations with lichenase (Megazyme, Warriewood, Australia). The enzymes used were  $\alpha$ -amylase (EC 3.2.1.1, human salivary, Type IX-A, Sigma), protease (from *Streptomyces griseus*, P-5130, Sigma), xylanase (EC 3.2.1.8, X-3876, Sigma), and hemicellulase (H-7649, Sigma). Extracts (3 mL) were treated with enzyme solution (20  $\mu$ L) after adjustment to appropriate pH and dilution with phosphate or acetate buffer (0.5M, 1 mL). Units of enzyme activity (Sigma units) used in digestions with these enzymes were 1.5, 4.4, 1.2, and 2.25 units, respectively; temperatures used in digestions were 25, 40, 25, and 40°C, respectively; and buffers were phosphate (pH 6.9), (pH 6.5), and (pH 7.5), and acetate (pH 4.5) and (pH 5.5), respectively.

### HPSEC

Except for NaOH-NaBH<sub>4</sub>, extracts were filtered (0.45- $\mu$ m nylon) without further preparation and put into autosampler vials which were then sealed. NaOH-NaBH<sub>4</sub> extracts were dialyzed before pH adjustment and filtration because vigorous outgassing created a foamy, difficult-to-mix mixture. NaOH-NaBH<sub>4</sub> extracts (3 mL) were dialyzed (2 hr, 12,000 MW cutoff) against 0.02% NaN<sub>3</sub> in water (10 L). Dialyzed extracts were centrifuged (45,000  $\times$  g, 10 min). All samples were injected (50–200  $\mu$ L) immediately upon being sealed in vials. The HPSEC system consisted of an inline degasser (Degasys DG-1200, Rannin), HP1050 series autosampler and two pumps (Hewlett Packard), columns (fractionation range to 20 million Da), UV/VIS spectrophotometer (1040M, Hewlett Packard), Dawn DSP-F multiple-angle laser light scattering (LS) detector (Wyatt Technology Corp, Inc.), differential refractive index (RI) detector (model 401, Waters, Millipore Corp.), PEEK mixing-T, and a fluorometry (FL) detector (model LS-5, Perkin Elmer). Column sequence was: guard (Waters, Millipore Corp), PL aquagel-OH 60 (7.5  $\times$  300 mm, Polymer Labo-

ratories), and Waters Ultrahydrogel 2000 (13  $\mu$ m, 7.8  $\times$  300 mm), 500 (10  $\mu$ m, 7.8  $\times$  300 mm), and 250 (6  $\mu$ m, 7.8  $\times$  300 mm). Mobile phase (0.02% NaN<sub>3</sub>) was pumped at 0.5 mL/min. Columns and RI detector were controlled at 35°C. Calcofluor reagent (100 mg/L, 0.1N NaOH) was pumped at 0.5 mL/min to the mixing-T before FL detection of  $\beta$ -glucans (Wood et al 1983, Suortti 1993). RI and LS (18 angles) output voltages were collected using Astra software which was used to calculate physical characteristics (MW, polydispersity, and root mean square radii). LS detector 11 (90°) outputs are plotted in the figures. Because only two auxiliary signals could be collected at one time in Astra, UV (280 nm) and FL (Ex 415, Em 445) outputs were switched to the computer as needed. FL and UV were used to detect elution regions for  $\beta$ -glucans and UV-absorbing, non- $\beta$ -glucan components, respectively.

### Requirements and Parameters for Calculations in Astra

Accurate measurement of eluting mass, normalization of LS detectors, delay between detectors, and LS detector constant are required for calculations. These parameters were determined as recommended in manufacturer's documentation. LS instrument constant ( $6.024 \times 10^{-6}$ ) was determined with toluene. Mass was provided by RI (range 4 $\times$ ) where the constant  $5.126 \times 10^{-6}$  (standard error  $\pm 3 \times 10^{-8}$ ) was determined using NaCl (dn/dc, 0.176). The definition of dn/dc is the proportional change in RI with change in concentration of a molecule. RI delay (0.113 mL) and normalization of LS detectors were determined using a P-20 Shodex P-82 pullulan standard (Millipore Corp.). UV (–0.111 mL) and FL (0.373 mL) delays were determined using fluorescein isothiocyanate-dextran (19,000 MW, Sigma). The dn/dc calculation method was used assuming a 0.145 dn/dc for  $\beta$ -glucans. Others (Williams et al 1991a, Wood et al 1991, Manzanares 1993) have assumed 0.13–0.16 dn/dc for  $\beta$ -glucans. A 0.6896% decrease in MW occurs for each 0.001 increase in dn/dc.

The degree of polynomial fit of line to LS data points in these plots was no greater than two out of a possible five. As generally accepted, Debye plots were used for molecules  $\geq 1$  million Da.

The performance of this system is demonstrated by the results determined for the pullulan P-200 standard. Recovery of injected standard was 99.5% and determined values ( $M_w = 18.62 \times 10^4$ ;

TABLE II  
Extraction (%) of  $\beta$ -Glucans from Waxbar Barley<sup>a</sup>

Extractant	0.5 hr	1.0 hr	2.0 hr	4.0 hr
Water				
23°C	39.6 $\pm$ 0.4	40.2 $\pm$ 0.4	41.3 $\pm$ 1.2 (23.2 $\pm$ 0.8) <sup>b</sup>	40.0 $\pm$ 0.8
65°C	56.7 $\pm$ 0.1	64.7 $\pm$ 2.0	65.9 $\pm$ 3.0 (55.5 $\pm$ 1.0)	62.4 $\pm$ 1.2
100°C	77.5 $\pm$ 1.9	78.5 $\pm$ 1.2	80.8 $\pm$ 1.4 (77.7 $\pm$ 1.1)	81.2 $\pm$ 2.5
NaOH (1N)				
23°C	99.9 $\pm$ 0.2	99.9 $\pm$ 0.3	99.9 $\pm$ 0.1	nd <sup>c</sup>
NaOH (1N) + NaBH <sub>4</sub> (1%)				
23°C	69.2 $\pm$ 0.2	69.1 $\pm$ 0.2	74.8 $\pm$ 1.5	nd
65°C	99.9 $\pm$ 0.1	100.0 $\pm$ 0.1	100.0 $\pm$ 0.1	nd

<sup>a</sup> Means  $\pm$  standard deviation,  $n = 3$ .

<sup>b</sup> Values in parentheses are for hot-ethanol-treated barley.

<sup>c</sup> Not determined.

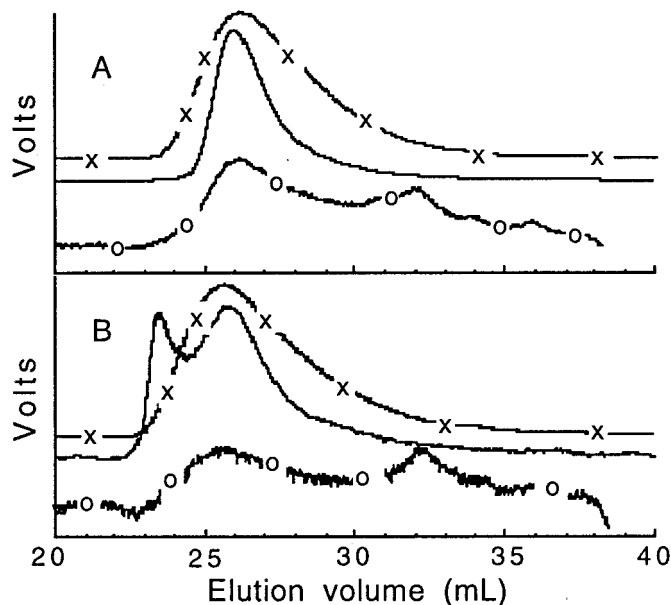


Fig. 1. High-performance size-exclusion chromatography (HPSEC) of NaOH-NaBH<sub>4</sub> extracts of Azhul (A) and Waxbar (B) barleys. Detectors were 90° angle light scattering (—),  $\beta$ -glucan and Calcofluor complex fluorometry (x), and refractive index (o). Voltage scales differed among detectors.

$M_w/M_n = 1.04$ ) were in good agreement with specifications provided by the manufacturer ( $M_w = 18.6 \times 10^4$ ;  $M_w/M_n = 1.13$ ).  $M_w$ ,  $M_n$ , and  $r_w$  are defined as weight-average molecular weight, number-average molecular weight, and weight-average root mean square radius, respectively. The  $M_z$  or z-average molecular weights are also given.  $M_z$  counts high molecular weight molecules more heavily than low averages ( $M_n$ ) or median averages ( $M_w$ ).  $M_w/M_n$  is a measure of polydispersity. MW is expressed as g/mol. The term "total  $\beta$ -glucan peak" means peak limits set at beginning and end of FI response to the  $\beta$ -glucan and Calcofluor complex.

### Compositional Analyses

Except for crude protein (Method 46-30, AACC 1995), proximate composition was determined using AOAC methods (AOAC 1990).  $\beta$ -glucan content of extracts and residues were determined by an enzymatic method (McCleary and Glennie-Holmes 1985) as described in the  $\beta$ -glucan assay kit (Megazyme, Warriewood, Australia).  $\beta$ -Glucanase activity in malt equivalents (U/kg) was analyzed by the method of McCleary and Shameer using a  $\beta$ -glucanase assay kit (Megazyme). Total dietary fiber was determined by the methods of Prosky et al (1988). Pentosans were determined as arabinose + xylose  $\times$  0.88. These sugars were measured after trifluoroacetic acid hydrolysis. The samples were digested with trifluoroacetic acid and the mixture evaporated to dryness (Olson et al 1988). The residues were dissolved in water. Anions and cations were removed by ion exchange (Amberlite MB-3 [H] [OH], Spectrum Chemical Co., Gardena, CA). Neutral sugars were separated by high-performance ion-exchange chromatography (Petersen et al 1984) using a lead form column (Supelcogel Pb, Supelco Inc., Bellefonte, PA). Moisture was determined as the difference in weight after heating at 130°C for 3 hr.

## RESULTS AND DISCUSSION

### $\beta$ -Glucanase Activity

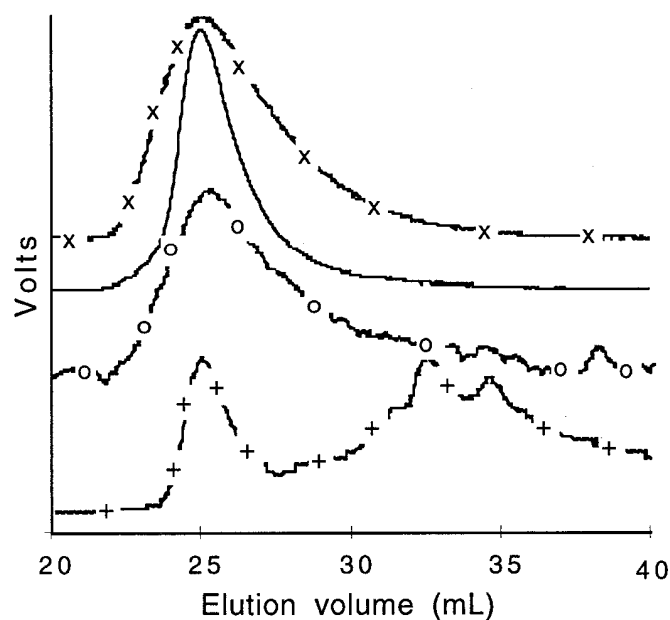
$\beta$ -Glucanase analysis ( $n = 3$ ) showed that Azhul, Waxbar, and enriched Waxbar contained  $82.9 \pm 2.0$ ,  $81.6 \pm 1.9$ , and  $84.4 \pm 0.7$  U/kg, respectively. Hot ethanol reduced these values to  $72.2 \pm 1.0$ ,

$67.4 \pm 0.3$ , and  $75.1 \pm 1.7$  U/kg, respectively. Because treated and untreated barleys had substantial  $\beta$ -glucanase activity, extracts for molecular characterization were prepared as quickly as possible and injected immediately into the HPSEC/MALLS system. The  $\beta$ -glucanase assay was also used to test 20- $\mu$ L aliquots of salivary  $\alpha$ -amylase, hemicellulase, protease, and xylanase.  $\beta$ -Glucanase activity for these enzyme solutions were 0, 170, 7, and 5 U/kg, respectively. Chromatographic results at the beginning and after 6 hr for an  $\alpha$ -amylase-treated solution of purified  $\beta$ -glucan (Megazyme) were the same, thus confirming low  $\beta$ -glucanase activity in the salivary  $\alpha$ -amylase.

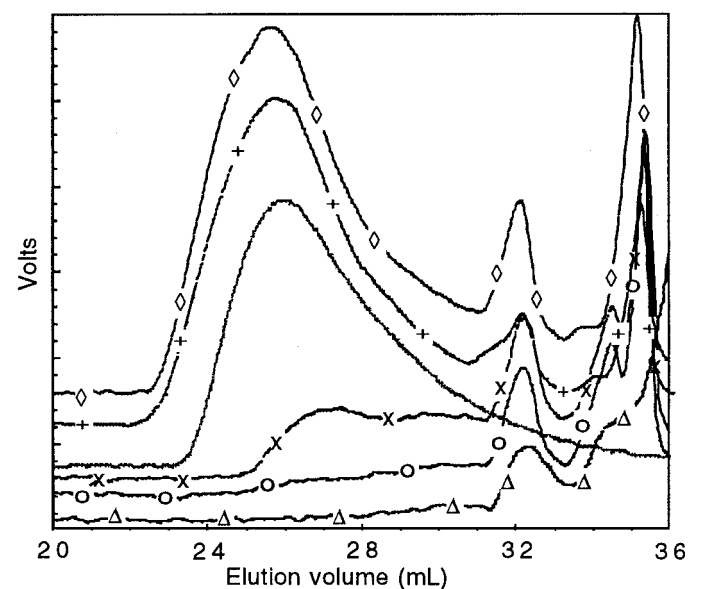
Total elimination of  $\beta$ -glucanase activity is difficult. Hot 75% ethanol treatment of oat bran reduced  $\beta$ -glucanase activity by 12%, the most of several treatments (Beer et al 1996). The difficulty in destroying this activity suggests that total destruction during meal preparation is unlikely. It could be expected that  $\beta$ -glucans consumed within cereal grains would degrade during passage through the intestine. Degradation of  $\beta$ -glucans during passage through the rat intestine has been demonstrated (Wood et al 1991). Others have reported that  $\beta$ -glucans isolated or in extracts were not sensitive to trypsin and attributed change in  $\beta$ -glucans in transit through the pig gut to microbes (Johansen et al 1993).

### Extraction

$\beta$ -Glucan analysis of Waxbar barley residues after 0.5, 1, 2, and 4 hr, showed that  $\beta$ -glucan extraction reached a maximum within 1 hr (Table II) under most extraction conditions. Water at 23°C (room temperature) extracted ~40% of the  $\beta$ -glucans. Second (65°C) and third (100°C) extractions with water increased the amount of  $\beta$ -glucan solubilized to 65 and 79%, respectively. When the barley was treated with hot ethanol, solubility of  $\beta$ -glucans with water at 23 and 65°C was lower. Extraction values are in the range of values reported for water solubility of barley  $\beta$ -glucans (Åman and Graham 1987, Bhatti 1993). A further extraction with NaOH at 23°C increased total  $\beta$ -glucan extraction to 98–100%. In the presence of oxygen, alkali catalyzes (in part) the transformation of reducing glucose units of polysaccharides to their C2-epimers (Whistler and BeMiller 1958). Addition of



**Fig. 2.** High-performance size-exclusion chromatography (HPSEC) of a water (100°C) extract of a  $\beta$ -glucan-enriched Waxbar barley fraction. Detectors were 90° angle light scattering (—),  $\beta$ -glucan and Calcofluor complex fluorometry (x), refractive index (o), and UV absorption (+). Voltage scales differed among detectors.



**Fig. 3.** Mass vs. elution volume for a hot water (100°C) extract of Waxbar barley with and without enzyme treatments. Refractive index was used for mass detection. Treatments were no enzyme ( $\diamond$ ),  $\alpha$ -amylase (+), lichenase (x),  $\alpha$ -amylase + lichenase (o), and  $\alpha$ -amylase + lichenase + hemicellulase ( $\Delta$ ).  $\beta$ -Glucan and Calcofluor complex fluorometry (—) in the no-enzyme extract is included for reference.  $\beta$ -Glucans were not present in any extracts treated with lichenase.

NaBH<sub>4</sub> to prevent alkaline degradation of polysaccharides (Forrest and Wainwright 1977) reduced total β-glucan extraction to ~70%. At 65°C, β-glucan extraction with NaOH-NaBH<sub>4</sub> was 100%. Forrest and Wainwright (1977) found little increase in β-glucan extraction when the temperature of NaOH-NaBH<sub>4</sub> was increased from 65 to 100°C. β-Glucan in Azhul barley seemed to be less soluble than that in Waxbar. At 1 hr, water (23°C) extracted 8.1% of the β-glucans from Azhul barley, and the additional extractions with water (at 65 and 100°C) and NaOH-NaBH<sub>4</sub> (65°C) increased solubilized β-glucans to 32.0, 42.7, and 95.8%, respectively.

Starch and pentosans are components that may contaminate β-glucan extracts. Analysis of residues indicate that 8–20% of the starch and 10–30% of pentosans was solubilized in water or alkali.

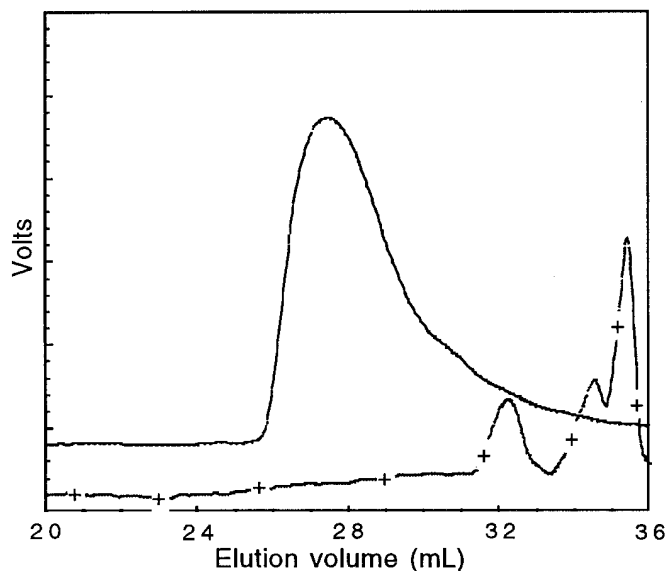


Fig. 4. Light scattering vs. elution volume for enzyme-treated hot water (100°C) extracts of Waxbar barley. Treatments were lichenase (—) and lichenase + α-amylase (+).

## HPSEC/MALLS

Chromatograms for NaOH-NaBH<sub>4</sub> (65°C) extracts of Azhul and Waxbar barleys are shown in Fig. 1. For both barleys, down-slopes of the β-glucan peaks (FL) were less acute than those of the up-slope, suggesting that some enzymatic degradation had occurred before extraction. Average  $M_w$  determined for total β-glucan peaks were  $3.30 \times 10^6$  and  $2.92 \times 10^6$  g/mol, respectively. These values exceed the range ( $1.7 \times 10^6$  to  $2.7 \times 10^6$  g/mol) previously reported for barley β-glucans (Wood et al 1991). β-Glucan polymers of up to  $4 \times 10^7$  Da containing firmly linked peptides are reported to exist in barley endosperm cell walls (Forrest and Wainwright 1977). In this study, the presence of peptide linkages with β-glucans was suggested by simultaneous detection of β-glucans (FL) and UV-absorbing material (Fig. 2). UV absorption could also be due to phenolic acids or other unidentified materials. UV absorption under the β-glucan peak was low, and it was not present in extracts of the same barley after several months of storage under normal conditions in the laboratory. Lack of absorbance after storage of the barley suggests that if any β-glucan-peptide complex exists, it is unstable, possibly due to endogenous enzymes.

Polydispersity for the β-glucan peaks (Fig. 1) of Azhul and Waxbar barley were high (2.23 and 2.85 mL, respectively) indicating a wide range of particle sizes. These particles could consist of multiple components both β-glucan and non-β-glucan in nature. The presence of multiple components is suggested by the LS detection of two peaks in the Waxbar extract (Fig. 1). The first peak (22.2–24.5 mL) may be the result of β-glucan aggregation or association of β-glucan to other polymeric components. In a similar type plot of unrefined heparin, the early eluting large mass fraction was attributed to aggregation (Wyatt 1993). Evidence of non-β-glucan components is shown using enzyme treatments of hot (100°C) water extracts of Waxbar barley. Hot water was used as extractant because of high β-glucan extraction with minimum difficulty in adjustment of pH for enzyme digestion. Treatment with salivary α-amylase had little effect on mass (RI) elution and had no discernible effect on β-glucan (FL) elution (Fig. 3). Lichenase greatly reduced mass in the 22–31 mL elution volume and totally eliminated the β-glucans (no FL response). Mass was reduced further when lichenase + α-amylase was used. Mass elution was not much changed (22–31 mL elution volume) compared

TABLE III  
Molecular Characteristics<sup>a</sup> of β-Glucans Sequentially Extracted from Azhul, Crystal, Prowashonupana, and Waxbar Barleys<sup>b</sup>

Barley	Extractant <sup>c</sup>	$M_n (\times 10^6 \text{ g/mol})$	$M_w (\times 10^6 \text{ g/mol})$	$M_z (\times 10^6 \text{ g/mol})$	$r_n (\text{nm})$	$r_w (\text{nm})$	$r_z (\text{nm})$	$M_w/M_n$
Azhul (ground)								
Flour	Water, 23°C	0.57	0.72	0.92	44	47	51	1.26
23°C residue	Water, 100°C	0.31	0.44	0.73	48	45	69	1.45
100°C residue	NaOH, 65°C	0.43	0.59	0.79	56	56	58	1.37
Crystal (ground)								
Flour	Water, 23°C	1.19	1.54	2.62	84	85	87	1.29
23°C residue	Water, 100°C	1.15	1.36	1.68	76	78	80	1.19
100°C residue	NaOH, 65°C	0.60	0.74	0.92	62	64	66	1.21
Waxbar (ground)								
Flour	Water, 23°C	0.95	1.57	2.28	36	40	45	1.64
23°C residue	Water, 100°C	1.21	1.26	1.31	37	38	38	1.04
100°C residue	NaOH, 65°C	0.44	0.74	1.56	43	47	73	1.66
Enriched Waxbar								
Flour	Water, 23°C	1.63	1.83	2.00	37	41	42	1.23
23°C residue	Water, 100°C	1.19	1.65	2.33	13	48	62	1.44
100°C residue	NaOH, 65°C	0.10	1.22	1.44	67	65	64	1.26
Prowashonupana								
Flour	Water, 23°C	1.85	2.34	3.18	91	114	147	1.27
23°C residue	Water, 100°C	0.75	1.02	1.87	84	108	157	1.36
100°C residue	NaOH, 65°C	0.16	0.54	2.21	54	59	60	3.43

<sup>a</sup>  $M_w$  = weight-average molecular weight,  $M_n$  = number-average molecular weight,  $M_z$  = z-average molecular weight;  $r_w$  = weight-average root mean square radius,  $r_n$  = number-average root mean square radius, and  $r_z$  = z-average root mean square radius.

<sup>b</sup> Means,  $n = 2$ . Azhul and Waxbar samples were treated with 75% ethanol at 80°C for 4 hr and dried before extraction. β-Glucan peak limits were set at 22 and 29 mL elution volume to prevent inclusion of an unknown component at 29–31 mL elution volume.

<sup>c</sup> All extracts were treated with salivary α-amylase. NaOH (1N) contained 1% NaBH<sub>4</sub>.

to lichenase +  $\alpha$ -amylase when treatments also included protease, xylanase, or hemicellulase, suggesting that pentosan and protein contributions in this region were small. (Chromatograms with protease and xylanase are not shown.) In alkali extracts, interference due to hemicellulose would be greater. The  $M_n$  for hemicelluloses extracted from barley with alkali ranged from  $1.55 \times 10^4$  to  $2.47 \times 10^5$  (Woolard et al 1977). None of the enzymes reduced the unknown mass component in the 29–31 mL elution volume. Light scattering in the 22–31 mL region of elution was high after lichenase treatment, but almost eliminated when  $\alpha$ -amylase was included (Fig. 4). The lichenase-resistant, amylase-labile material (starch) had a very high molecular weight. Molecular weights ( $M_n$ ,  $M_w$ ,  $M_z$ ) for this material in Azhul were  $7.6 \times 10^6$ ,  $8.5 \times 10^6$ , and  $9.3 \times 10^6$  g/mol, respectively, and in Waxbar they were  $1.5 \times 10^6$ ,  $2.3 \times 10^6$ , and  $3.2 \times 10^6$  g/mol, respectively. Because starch elution overlaps  $\beta$ -glucan elution, and its capacity for LS is high, an amylase digestion should be included in methods for molecular characterization of barley  $\beta$ -glucans. Digestion with  $\alpha$ -amylase was included in the method for characterizing oat  $\beta$ -glucans (Beer et al 1995).

Comparisons of sequential extracts ( $\alpha$ -amylase-treated) of Waxbar showed that MW decreased after the first water extraction (23°C) and that MW for water extracts at 65 and 100°C were similar. Therefore, with the greater extraction at 100°C, further comparisons were made using extracts with water at 23, 100, and NaOH-NaBH<sub>4</sub> (Table III). Differences in molecular weights of  $\beta$ -glucans existed among barley cultivars and among extracts (Table III).  $\beta$ -Glucans extracted with water at room temperature (23°C) had higher molecular weights than those extracted with water at 100°C and with alkali. The lower molecular weights of  $\beta$ -glucans in the 100°C water and alkali extracts may result from the  $\beta$ -glucanase activity or mechanical damage occurring during the additional extraction steps. Degradation may explain the reason average  $M_w$  values were generally lower than the  $1.7 \times 10^6$  to  $2.7 \times 10^6$  g/mol previously reported for barley  $\beta$ -glucans (Wood et al 1991).

## CONCLUSIONS

Hot-alcohol treatment did not destroy  $\beta$ -glucanase activity and affected  $\beta$ -glucan extraction. Combinations of enzyme treatments of extracts indicate that starch, unless removed by  $\alpha$ -amylase, interferes with measurement of physical characteristics of  $\beta$ -glucans. Although present in extracts, pentosan interference in the region of  $\beta$ -glucan elution (22–29 mL) was not demonstrated by treatments with combinations of enzymes. A single extraction with hot water is best because there is greater extraction with hot water and the MW of  $\beta$ -glucans decreases with sequential extraction where enzymatic or mechanical degradation occur. There appear to be molecular differences in  $\beta$ -glucans among barley varieties, although differences could reflect storage history whereby enzymatic degradation differed. Although commercially produced barley is generally not stabilized, barley for food use should be stabilized as soon as possible to reduce  $\beta$ -glucanase activity and retain maximum benefit of the  $\beta$ -glucans.

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[Received October 15, 1996. Accepted May 5, 1997.]