Solution and Conformational Properties of Wheat β-D-Glucans Studied by Light Scattering and Viscometry

Wei Li,[†,‡] Steve W. Cui,*[,†] and Qi Wang[†]

Food Research Program, Agriculture and Agri-Food Canada, Guelph, N1G 5C9 Ontario Canada, and Department of Food Science, University of Guelph, Guelph, N1G 2W1 Ontario, Canada

Received August 30, 2005; Revised Manuscript Received October 11, 2005

The solution properties of wheat β-glucan were investigated by light scattering and viscometric methods. The hydrodynamic radius (R_h), weight average molecular weight (M_w), radius of gyration (R_g), and the second virial coefficient (A_2) of wheat β-glucan were determined by both dynamic and static light scattering methods, whereas the critical concentrations (c^*) of the solution were derived from [η] via viscometric method. The structure sensitive parameters, ρ (1.52−1.62), the conformation parameter ν (0.62), and the Mark−Houwink−Sakurada exponents α (0.78) confirmed the random coil conformation of wheat β-glucan in 0.5 M NaOH solution. The characteristic ratio (4.97) was obtained by the random flight model, and the statistical segment length (8.83 nm) was derived from the wormlike cylinder model. It was found that the wormlike cylinder model could explain the chain stiffness better than the random flight model, which suggested an extended random coil conformation of wheat β-glucan in 0.5 M NaOH solution. The study also revealed that the structure feature of wheat β-glucan; that is, the higher trisaccharide-to-tetrasaccharide ratio contributed to the stiffer chain conformation compared with other cereal β-glucans.

1. Introduction

Mixed linked (1→3) (1→4)-β-D-glucans are found in cereal endosperm cell walls. The main sources of β-D-glucans are from oat, barley, wheat, and rye, and the amounts in oat and barley are more than in wheat and rye. Research interest in cereal β-glucans is largely due to their beneficial physiological effects on human health and important physical properties for the food industry. β-D-Glucans are attributed to reducing serum cholesterol and glucose levels in humans.[1,2,3] Their rheological properties, such as high viscosity and the ability to form a gel, made cereal β-glucans potentially useful in the food industry as natural hydrocolloids.[4]

The primary structure of β-D-glucan is a linear chain composed of only D-glucopyranose, which is arranged as blocks of consecutive (1→4)-linked β-D-glucosyl residues separated by single (1→3)-linkages. The β-(1→3)-linked cellotriosyl and cellotetraosyl units are over 90% of the polymer chain; however, there is evidence for a minor amount of β-(1→4)-linked cellulose like blocks which have more than 4 residues and up to 14.[5] The presence of (1→3)-linkages prevents crystallization of otherwise long cellulosic chain and bestows apparent water solubility on the cereal β-glucan.[6] Significant structural differences among cereal β-glucans are characterized by the trisaccharide-to-tetrasaccharide ratios, which follows the order of wheat (4.2−4.5), barley (2.8−3.3), and oat (2.0−2.4).[7] Compared with other cereal β-glucan, the higher tri/tetra ratio of wheat β-glucan gives it a higher structural regularity of its backbone chain, which is believed to make it easier to form ordered structures, such as junction zones, between molecules and, hence, a higher possibility to form a gel.

The primary structure and physical properties of cereal β-glucans have been subjected to many investigations. However, little information has been obtained on the structure-function relationship of cereal β-glucans. Lazaridou et al.[8] studied the effects of fine structure and molecular size on the rheological properties, while the mechanism of how the structure of β-glucan influences its physical properties has remained unresolved. Since the conformational properties of polysaccharides are dictated by their primary structure and physical properties are closely related to the conformational properties, the study of conformational properties of cereal β-D-glucans are important to understand their structure−function relationship.

One of the most important experimental techniques for determining dilute solution behavior of polysaccharides is light scattering. When light scattering techniques and viscometry measurements are combined, weight average molecular weight, radius of gyration, hydrodynamic radius, and intrinsic viscosity can be determined, which provide a basis for studying the solution properties of polysaccharides. Unfortunately, when cereal β-D-glucan is dissolved in water, aggregates are inevitably present due to the strong intermolecular hydrogen bonding. The aggregation phenomenon will lead to inaccurate determination of molecular weight and conformation parameters. A method to eliminate aggregates of cereal β-glucan in dilute solutions was developed by Li et al.[9] using 0.5 M NaOH solutions, which allows a reliable measurement of conformational properties by static and dynamic light scattering methods.

Several studies on the conformational properties of oat and barley β-D-glucans have been reported.[6,10−14] However, no conformational information of wheat β-D-glucan has been reported, and the relationship between the structure and conformation of cereal β-D-glucans has not been discussed. To our best knowledge, only one study was reported using the combination of static and dynamic light scattering and viscometry to determine solution properties of cereal β-D-glucan.[6] However, no conformational information was derived due to aggregation phenomenon in solutions. The objective of the present study was to investigate the conformational properties

* Corresponding Author. Tel.: (+1) 519-780-8028. Fax: (+1) 519-829-2600. E-mail address: cuis@agr.gc.ca.
† Agriculture and Agri-Food Canada.
‡ University of Guelph.

10.1021/bm050625v CCC: $33.50
Published on Web 01/11/2006

of wheat β-D-glucans using light scattering and viscometric methods. The structure−conformation relationship of cereal β-D-glucans will be discussed by comparing our result to the results of other cereal β-D-glucans studies from the literature.

2. Experimental Section

2.1. Materials. Wheat β-D-glucan was extracted in alkaline condition from white wheat bran powder 50 (50 mesh, Hayhoe Mills Ltd., Canada) using the procedure of Cui et al.[15] and further purified by repeated ammonium sulfate precipitation. Purified wheat β-D-glucan (91.58% purity) was fractionated into six fractions differing in molecular weight using the gradient ammonium sulfate precipitation method described previously[16] with some modifications. Six fractions (from F1 to F6) were precipitated from solutions at the ammonium sulfate concentrations of 16.26%, 17.01%, 18.01%, 19.44%, 24%, and 40%.

2.2. Sample Preparation. To prevent aggregation of wheat β-glucans, a 0.5 M NaOH solution was used as the solvent for light scattering and viscometric measurements.[9] Wheat β-D-glucan NaOH solutions (0.075%, w/v) were prepared by gentle stirring for 30 min at room temperature. All of the solutions were filtered through a 0.45μm nylon syringe filter (Chromatographic Specialties Inc, USA).

2.3. Apparatus of Light Scattering. A 35 mW helium neon laser (Melles Griot Laser Group, Carlsbad, CA) with wavelength of 632.8 nm was focused on a precision scattering cell (diameter 25 mm) containing a sample solution. Both static and dynamic measurements were conducted using a Brookhaven light scattering instrument including a precision Goniometer, a photomultiplier, and a 128-channel BI-9000AT digital autocorrelator (Brookhaven Instruments, Holtsvile, NY). Total intensity light scattering measurements were conducted in the angular range of 30°−140° (for dynamic light scattering, at 90° only). Toluene was used as a reference with the Rayleigh ratio of 1.398×10^{-5} cm^{-1} in the static light scattering measurements. The refractive index increment, dn/dc, was chosen as 0.146 mL g^{-1} for β-D-glucan in 0.5M NaOH solution.[17] The particle size distributions were calculated by the constrained regularization (CONTIN) method using Brookhaven dynamic light scattering software. All light scattering measurements were performed at 25 °C.

2.4. Static Light Scattering (SLS). Static light scattering allows the determination of weight average molecular weight M_w, radius of gyration R_g, and the second virial coefficient A_2 using the following equation:

$$Kc/R_\theta = 1/M_w + 1/3(R_g^2/M_w)q^2 + 2A_2c \quad (1)$$

where K is an optical contrast factor, c is the polymer concentration, R_θ is the Rayleigh ratio (normalized scattering intensity), and the scattering vector is defined as

$$q = (4\pi/\lambda) \sin \theta/2 \quad (2)$$

with $\lambda = \lambda_0/n_0$, the wavelength of the light in a medium of refractive index n_0, λ_0, the wavelength in a vacuum. The optical contrast factor K is defined by

$$K = 2\pi^2 n_0^2 (\mathrm{d}n/\mathrm{d}c)^2/(N_0\lambda_0^4) \quad (3)$$

where dn/dc is the refractive index increment of the solution and N_0 is Avogadro's number. The Zimm method is a graphical technique to extrapolate simultaneously Kc/R_θ to zero angle and infinite dilution. In a Zimm plot, Kc/R_θ is plotted as a function of $q^2 + kc$, where k is an arbitrary chosen constant. The slope of the angular dependence at $c = 0$ corresponds to the z-average mean square radius of gyration $(R_g)^2$. A_2 can be calculated from the slope of concentration dependence at $\theta = 0$, and $1/M_w$ is obtained from the intercept of both $c = 0$ and $\theta = 0$ extrapolated lines.

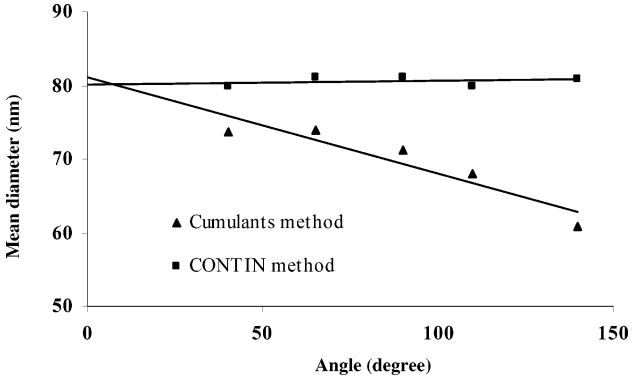

Figure 1. Angular dependence of the mean diameters of wheat β-glucan fraction 1 measured in 0.5 M NaOH solution by DLS.

2.5. Dynamic Light Scattering (DLS). The measured intensity autocorrelation function $G^{(2)}(\tau)$ is related to the electric field autocorrelation function $g^{(1)}(\tau)$ by the Siegert relation

$$G^{(2)}(\tau) = A[1 + \beta|g^{(1)}(\tau)|^2] \quad (4)$$

where A is the baseline constant, β is the coherence factor, which is generally considered as an adjustable parameter in the data analysis procedure, and τ is the correlation time. For continuous distribution of decay rate Γ

$$g^{(1)}(\tau) = \int_0^{+\infty} G(\Gamma) \exp(-\Gamma\tau)\, \mathrm{d}\Gamma \quad (5)$$

where $G(\Gamma)$ is a continuous distribution function of decay rates, which is correlated with $g^{(1)}(\tau)$ by a Laplace transformation. The decay rate is defined as $\Gamma = K^2D$, where D is the diffusion coefficient and K is the optical contrast factor. The hydrodynamic radius (R_h) can be calculated by applying the Stokes−Einstein relation

$$R_h = kT/6\pi\eta D \quad (6)$$

where T is the absolute temperature, k is the Boltzmann constant, and η is the solvent viscosity. In the case of a polydisperse solute, the distribution of the diffusion coefficient, and thus the hydrodynamic diameter distribution function, is obtained from $g^{(1)}(\tau)$ by inverse Laplace transformation using constrained regularization method (CONTIN) method.

2.3. Intrinsic Viscosity Measurement. The intrinsic viscosities were determined by dilute solution viscometry using a Cannon Ubbelohde Dilution B glass viscometer (size 50, 0.8−4 cSt; Glass Artifact Viscometers, Braintree, Essex, U.K.) immersed in water bath at a constant temperature of 25 °C. Wheat β-glucan concentrations were selected in the range that the relative viscosity, η_r, was kept from 1.2 to 2.0, so that no end effect correction is necessary. Intrinsic viscosity $[\eta]$ was calculated using the following relationship:

$$[\eta] = \lim_{c\to 0} (\eta_{sp}/c) = \lim_{c\to 0} (\ln \eta_r/c) \quad (7)$$

where c is the concentration of polymer, η_r is the relative viscosity, and η_{sp} is the specific viscosity, defined as $\eta_r - 1$. Huggins-Kramer plots of η_{sp}/c and $\ln(\eta_r)/c$ versus c were then used to estimate the intrinsic viscosity $[\eta]$ by extrapolation to zero concentration.

3. Results and Discussion

3.1. Results.

3.1.1. Dynamic Light Scattering. Figure 1 shows the apparent mean diameters of wheat β-glucan fraction 1 in 0.5 M NaOH solution measured at different angles and analyzed by both the CONTIN and the cumulants methods. The results

Table 1. Experimental Data of Static and Dynamic Light Scattering of Wheat β-Glucan Fractions[a]

	M_w (g/mol)	A_2 (cm^3mol/g^2)	R_g (nm)	R_h (nm)	R_g/R_h
F0	328 700	1.04×10^{-3}	45.6	26.2	1.74
F1	591 300	1.13×10^{-3}	59.5	36.7	1.62
F2	475 200	1.17×10^{-3}	45.4	28.5	1.59
F3	367 900	1.24×10^{-3}	37.0	24.3	1.52
F4	277 800	1.43×10^{-3}	31.0	19.8	1.57
F5	194 200	1.42×10^{-3}	26.5	16.8	1.58
F6	54 600	1.10×10^{-3}	12.6	7.8	1.61

[a] F0 is unfractionated wheat β-glucan. Molecular weight (M_w), the second virial coefficient (A_2), and radius of gyration (R_g) were measured by SLS; the hydrodynamic radius (R_h) was measured by DLS.

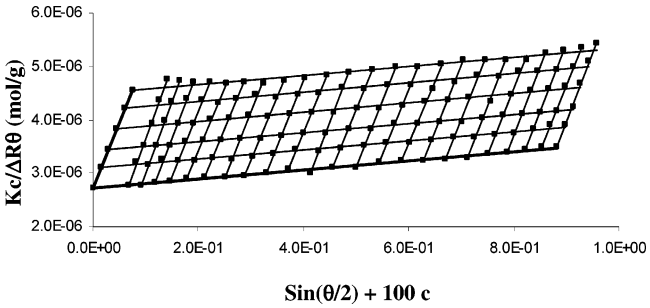

Figure 2. Zimm plot of wheat β-glucan fraction 3 by SLS measurement.

analyzed by the cumulants method showed angular dependence, whereas the CONTIN method did not. For the former approach, when the angle was extrapolated to zero, the mean diameter obtained is in good agreement with the result analyzed by the CONTIN method (Figure 1). Fraction 1 is the sample of largest size among the group. Other fractions showed either similar or less angular dependence. By using the CONTIN method, the hydrodynamic radius of each fraction of wheat β-glucan at a specific angle 90° was calculated and summarized in Table 1.

3.1.2. Static Light Scattering. The Zimm plot of a fraction of wheat β-glucan (F3) measured by SLS in 0.5 M NaOH solution is presented in Figure 2 as an example. The absolute weight average molecular weight (M_w), the radius of gyration (R_g), and the second virial coefficient (A_2) for all of the samples were calculated from the Zimm plots (Table 1). The molecular weight decreases significantly from F1−F6, and the values of each fraction are in good agreement with the results measured by size exclusion chromatography.[18] In the extreme dilute solution, the second virial coefficient (A_2) is a quantitative indicator of the affinity between the polymer and the solvent, i.e., the thermodynamic quality of the solvent for the given polymer.[19] The high positive value of A_2 for each fraction suggests that the 0.5 M NaOH solution is a good solvent for wheat β-glucan. The radius of gyration, a geometrical parameter of a polymer chain, is given by a sum over all distances of the scattering elements from the center of mass or the sum over all intramolecular distances in the macromolecule.[20] The values of R_g increased with molecular weight and were higher than that of R_h. The relationship between R_g and M_w or R_h can be used to interpret the shape of wheat β-glucan chains in solutions.

3.1.3. Intrinsic Viscosity. Intrinsic viscosity [η] of wheat β-glucans in water and in 0.5 M NaOH solution were estimated from Huggins−Kramer plot by extrapolation to zero concentration and the results are summarized in Table 2. The higher values in aqueous solution indicate the presence of aggregates, which gives higher apparent molecular weight of wheat β-glucan fractions. The intrinsic viscosities obtained in 0.5 M NaOH are more close to the real intrinsic viscosities for these β-D-glucan fractions.

Table 2. Intrinsic Viscosity of Wheat β-Glucans Measured by Capillary Viscometer[a]

	intrinsic viscosity (dL/g)	
	in water	in 0.5 M NaOH solution
F0	4.55 ± 0.07	3.47 ± 0.17
F1	6.50 ± 0.00	5.50 ± 0.39
F2	6.10 ± 0.14	4.71 ± 0.17
F3	5.15 ± 0.21	3.93 ± 0.10
F4	3.70 ± 0.14	2.69 ± 0.17
F5	2.80 ± 0.00	2.15 ± 0.00
F6	1.49 ± 0.01	0.88 ± 0.06

[a] $n = 2$.

3.2. Discussion.

3.2.1. Data Analysis for DLS. Many interpretation methods for autocorrelation functions of DLS measurements have been developed based on different algorithms, such as the cumulants method, histogram analysis, constrained regularization (CONTIN), nonnegatively constrained least-squares method (NNLS), etc. Each method was designed to solve a specific problem, and thus, each has both advantages and disadvantages. The results calculated by different analytical methods might be different, and sometimes the difference is significant for some samples, but not for others. Therefore, it is critical to find a suitable method analyzing the results of DLS measurements.

A comparison of these analytical methods was well studied by Stock and Ray.[21] The cumulants method was the most commonly applied method for polydispersity analysis in the early years for dynamic light scattering. It provides accurate results when the cumulants series converges; therefore, it is suitable for the distributions which only have small values of high-order moments. The limitations are that the result is sensitive to the initial guess and it does not provide the explicit distribution of the diffusion coefficient. The CONTIN method can yield accurate values of the average and variance in most cases, because there is no initial guess required and dust is accounted for. It is the most popular method at present. However, when data have significant noise levels, the bimodal distributions obtained tend to be overly smoothed. The nonnegatively constrained least-squares method (NNLS) is best used for multimodal distributions with narrow, widely spaced peaks, which gives accurate estimates of the average and variance. Disadvantages of this method are that it often obtains false peaks and tends to overcompensate for dust.[21]

Wheat β-D-glucan fractions in 0.5 M NaOH solution are free of aggregates and have a narrow unimodal distribution.[9] As discussed above, the NNLS method is not preferred compared to the CONTIN method in this case. However, when the aggregation phenomenon of wheat β-D-glucan in water is investigated, the NNLS method becomes the first choice.[9] As shown in Figure 1, the CONTIN and cumulants methods gave the consistent results. The cumulants method would fail in the presence of dust and the dust cannot be completely avoided for each sample even though special caution is taken for removing dust during the sample preparation. Furthermore, the CONTIN method fits the correlation functions best by giving smaller error values (data not show). As a result, the CONTIN method was chosen to calculate the hydrodynamic radius of wheat β-D-glucan samples.

3.2.2. Influence of Fractionation Methods on Molecular Structures. For studying conformational properties of cereal β-D-glucans, it is essential to obtain β-D-glucan fractions which

have homogeneous structure but different molecular weight. Reported fractionation methods include ultrasonic irradiation,[22] acid hydrolysis,[23] enzyme hydrolysis (lichenase[10,24] and cellulase[11] degradation), and gradient ammonium sulfate precipitation.[16,25] Most methods are based on the partial degradation mechanism of β-D-glucan molecules to obtain different molecular weight fractions, except the gradient ammonium sulfate precipitation method, which is based on molecular weight effect of cereal β-D-glucans on their solubility. Fractionation methods may cause the structural changes of cereal β-D-glucans, which may influence their conformational properties. For example, the degradation method may change the primary structure of β-D-glucans especially for the small molecular weight fractions. The changes include change of tri/tetra ratio, loss of long cellulose like segments, etc. The degradation methods usually lead to a high value of polydispersity index of β-D-glucan fractions. The theoretical value of the polydispersity index is given as 2.0 for random degradation of infinite chains.[26] Although the reported values of degraded fractions are lower than the theoretical value (1.4−1.9),[11,24,27] data derived from SLS and DLS measurements on highly polydisperse fractions are not appropriate for the prediction of molecular conformation. The degradation of ultrasonic irradiation and acid hydrolysis likely occurs at the weak points of β-D-glucan chains rather than at random points; however, the exact cleaving positions in the polymer chains are difficult to determine. Thus, even though these two degradation methods may cause preferred structural changes of cereal β-D-glucan, the detailed structural changes remain unknown. Enzymatic degradations attack the specific sites of the substrate according to the affinity of the enzyme. For example, cellunase prefers to attack the consecutive (1→4)-linkages,[11] which leads to a decrease in the long cellulose like segments and cellotetraosyl segments, which may cause the increase of cellotriosyl unit proportion. The higher trisaccharide proportion increases the chances of forming the consecutive cellotriosyl segments, which could result in a rigid chain conformation. Therefore, the molecular chains of fractions obtained by partial cellulase degradation could be stiffer than that of the original β-D-glucans. This theory is supported by the results of Roubroeks et al. on oat β-D-glucan.[11]

Lichenase specifically cleaves the (1→4)-linkage of the 3-O-substituted glucose unit in β-D-glucans.[28] As a result, most of the major components along β-D-glucan chains, i.e., trisaccharide segments, tetrasaccharide segments, and long cellulose like segments etc., remain intact after lichenase degradation. It is likely that the changes in structural features after lichenase degradation could be the least compared with other degradation methods.

Fractionation by the gradient ammonium sulfate precipitation method is based on the solubility differences in ammonium sulfate solutions of different molecular weight β-D-glucans. Because there are no chemical reactions and physical degradation occurred during the fractionation process, Wang et al.[16] and Li et al.[18] proved that fractions obtained by this method exhibited the same structure features as the original sample. However, the reported values of the polydispersity index for fractions obtained by gradient ammonium sulfate precipitation method were much lower (F1, 1.26; F2, 1.19; F3−F6, <1.1).[18] This allows us to obtain more precise results for conformation studies of cereal β-D-glucans. Therefore, this fractionation method was chosen in the present study.

3.2.3. Prediction of Critical Concentration. Intrinsic viscosity can be used to estimate the critical concentration, c^*, defined as the concentration at which molecular entanglement begins.

Table 3. Critical Concentrations (c^*, c^{**}) of Wheat β-Glucans Calculated from Their Intrinsic Viscosities

	in water		in 0.5 M NaOH solution	
	c^* (g/dL)	c^{**} (g/dL)	c^* (g/dL)	c^{**} (g/dL)
F0	0.13	0.46	0.17	0.86
F1	0.09	0.49	0.11	0.54
F2	0.10	0.58	0.13	0.63
F3	0.11	0.66	0.15	0.76
F4	0.16	0.81	0.22	1.11
F5	0.21	1.07	0.27	1.39
F6	0.22	1.42	0.38	2.41

Determination of c^* via rheological measurements requires large quantities of samples and it is extremely time-consuming to prepare fractions in such quantities. Morris[29] suggested that $c^* \approx 4/[\eta]$ for most polysaccharides, based on the result of Robinson et al.[30] in a study of guar galactomannans. Doublier and Wood[23] studied the solution behavior of oat β-glucan and gave $c^* \approx 2.5/[\eta]$. These two formulas were compared by Burkus and Temelli[31] on the study of barley β-glucan, and they suggested that the formula of $c^* \approx 2.5/[\eta]$ was more suitable for predicting the critical concentration of β-glucan based on the fact that viscosity at c^* is about 10 mPa s in all cases. However, there are two concerns when applying the formula of $c^* \approx 2.5/[\eta]$ to wheat β-glucan. First, the intrinsic viscosity used by Doublier and Wood[23] was not measured directly, instead it was calculated from the Mark-Houwink-Sakurada equation by assuming $\alpha = 0.723$. Second, the molecular weight of oat β-glucan used by Doublier and Wood[23] was 1 200 000 g/mol, which is much higher than those of wheat β-glucan in the present study. When the molecular size decreases to a certain level, only considering the volume occupancy to predict the critical concentration of polymers may not be adequate due to the increasing mobility of small molecules in solution. The results of Vaikousi et al.[32] on the study of barley β-glucan and Lazaridou et al. on the study of oat β-glucan revealed that the value of $c^*[\eta]$ decreased when the molecular weight was less than 150 000 but kept the same value for the larger molecules ($M_w > 150\,000$). Lazaridou et al.[8] reported $c^*[\eta] \approx 0.59$ and $c^{**}[\eta] \approx 2.99$ for wheat β-glucan ($M_w = 209\,000$), which was consistent with the values of oat and barley samples.[32,33] The second critical concentration (c^{**}) is defined as the concentration at which the solution transits from semidilute to concentrated domain. The presence of the intermediate zone (semidilute domain) between the dilute ($c < c^*$) and concentrated ($c > c^{**}$) domains suggested a rigid rodlike conformation of polymers.[34] Therefore, the formulas of $c^*[\eta] \approx 0.59$ and $c^{**}[\eta] \approx 2.99$ were used for wheat β-glucan and its fractions (except F6). Due to high mobility of fraction 6, a specific formula with a value of $c^*[\eta] \approx 0.33$ and $c^{**}[\eta] \approx 2.12$ was adapted from barley β-glucan fraction, BGL70,[33] which had a similar molecular weight to F6 of wheat β-glucan. Based on these equations, the critical concentrations of wheat β-glucans in pure water and in 0.5 M NaOH solution were calculated and summarized in Table 3. These predicted data provide a useful reference for studying the solution and gelation properties of wheat β-glucans.

3.2.3. Determination of the Shape of Wheat β-D-Glucan in Solutions. The study of the relationship between the radius of gyration, intrinsic viscosity, hydrodynamic radius, and molecular weight could provide some general information about the shape and conformation of polysaccharide chains. The structure sensitive parameter ρ, conformation parameter ν, and the Mark−Houwink−Sakurada exponent α were obtained as

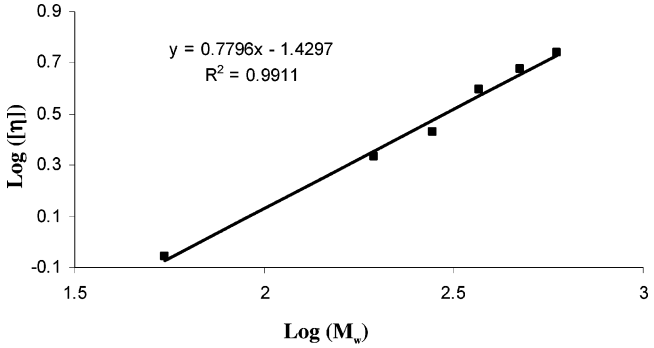

Figure 3. Double logarithmic plot of [η] against M_w of wheat β-glucan in 0.5 M NaOH solution.

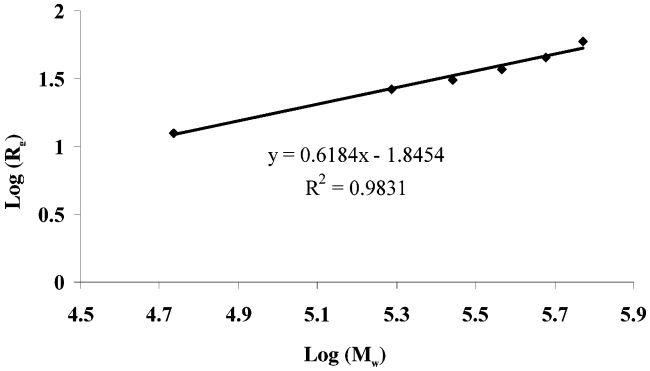

Figure 4. Double logarithmic plot of the molecular weight vs the radius of gyration of wheat β-glucan in 0.5 M NaOH solution.

described below. These three parameters are good indicators of the molecular shape of wheat β-glucans in solutions.

3.2.3.1. Structure Sensitive Parameter ρ. By combining the static and dynamic light scattering results, $\rho = R_g/R_h$ can be calculated. ρ is an important parameter for characterizing polymers and colloids It depends on the chain architecture, conformation and polydispersity, but not on the molar mass.[35] For linear, flexible chains in an unperturbed state, i.e., in θ solvent, the Kirkwood−Riseman theory gives a ρ value of 1.50.[35] Burchard suggested more explicit values: for monodisperse random coils, $\rho = 1.50$ in a θ solvent, and $\rho = 1.78$ in a good solvent.[20] The ρ values of each fraction of wheat β-glucan are in the range of 1.52−1.62, which are lower than that of the original wheat β-glucan sample ($\rho = 1.74$). This is expected, since the fractions have a much narrower size distribution (polydispersity, 1.03−1.26) compared to the original sample (polydispersity, 1.65).[18] Comparing the ρ values of each fraction with the suggested value by Burchard,[20] a random coil conformation of wheat β-glucan in dilute solution is confirmed.

3.2.3.2. Mark−Houwink−Sakurada Exponent α. Intrinsic viscosity is usually related to molecular weight by the Mark−Houwink−Sakurada equation

$$[\eta] = KM_w^{\alpha} \tag{8}$$

where α is the Mark−Houwink−Sakurada exponent. In particular, if a polymer is a rod, α is ~1.8, whereas, for a random flight flexible coil, it is usually assumed to lie in the range of 0.5−0.8.[30] Teraoka[36] suggested that the value of α was around 0.7−0.8 for flexible chains in a good solvent, and exceeds 1 for rigid chains. In a θ solvent, a flexible chain has $\alpha = 0.5$. Apparently, α is higher for a more extended conformation, because a polymer molecule with a greater dimension for a given contour length will experience more friction to move in the solvent.[36] In a good solvent, polymer−solvent interaction is preferred in solution, which favors the extension of polymer chains to interact with solvent resulting in a more rigid conformation. From the double logarithmic plot of [η] against M_w (Figure 3), the value of α for wheat β-D-glucan was obtained as 0.78 in 0.5 M NaOH solution. This result again confirms the random coil conformation of wheat β-glucan in solution. The value of α for wheat β-glucan is in good agreement with $\alpha = 0.71$ for barley β-glucan,[12,37] $\alpha = 0.72$ for β-glucan isolated from beer,[6] and $\alpha = 0.82$ and 0.73, for oat β-glucan.[11,13]

3.2.3.3. Conformation Parameter ν. The dependence of the radius of gyration on the molecular weight is described by a power-law behavior[20]

$$R_g = KM^{\nu} \tag{9}$$

where the exponent ν is a characteristic parameter for a molecular architecture. The exponent ν can vary from 0.33 for hard spheres up to 1.0 for rigid rods. The ν value for random coils is between 0.5 and 0.6. For a linear random coil, ν equals 0.60 in good solvent and 0.50 in θ conditions.[20] The double logarithmic plot of the molecular weight versus the radius of gyration of wheat β-glucan is shown in Figure 4. The ν value of 0.62 was obtained from the slope of the correlation curve, which indicates wheat β-D-glucan exhibits a random coil conformation in good solvent. The higher value than 0.6 of wheat β-glucans implies the stiffer conformations than that of the typical random coil. This ν value is consistent with that of oat β-D-glucan (0.56−0.70).[10]

3.2.4. Estimation of Macromolecular Dimensions. Based on two macromolecular models, the results of intrinsic viscosity and molecular weight were used to characterize the chain stiffness of wheat β-D-glucan by calculating the unperturbed dimensions of the chains in the random flight model and the statistic segment length of the macromolecule in the wormlike cylinder model (semi-flexible chain model).

3.2.4.1. Random Flight Model. In the random flight model, a polymer chain is considered as a collection of stiff rods connected by totally flexible universal joints.[30] The average root-mean-square end to end distance (L') is given as

$$L' = n^{1/2}l \tag{10}$$

where n is the number of rod and l is the length of each rod. The measured value of end to end distance (L) at unperturbed condition is always greater than L' due to the energetically constrained joints (dihedral angles ϕ,ψ for polysaccharides) and the excluded volume effect, namely, two or more segments along the chain cannot occupy the same volume element at the same time.

The random flight treatment neglects the excluded volume effect, because this effect becomes small with decrease in molecular size. The characteristic ratio (C_∞), a measure of the restriction of chain flexibility, is defined as

$$C_\infty = L^2/L'^2 \tag{11}$$

The intrinsic viscosity ([η]), molecular weight (M), and L' can be related by the Flory−Fox equation

$$[\eta] = \Phi L^3/M \tag{12}$$

where Φ is a constant ($\approx 2.6 \times 10^{21}$ mol^{-1} for random coils). From eqs 11 and 12, we have

$$L^2 = C_\infty l^2 n = C_\infty l^2 M/m \tag{13}$$

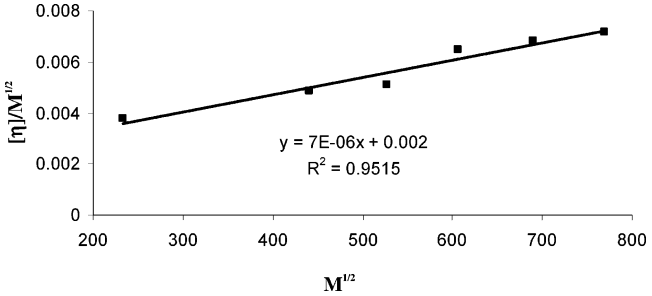

Figure 5. BSF plot of wheat β-glucan in 0.5 M NaOH solution.

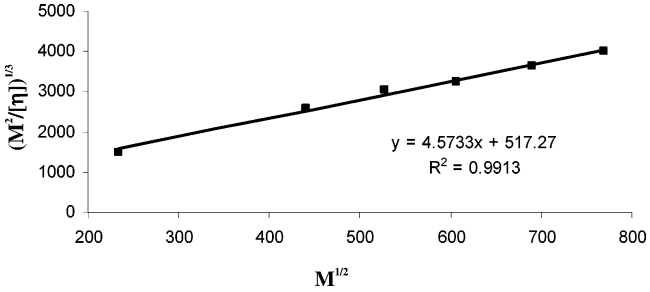

Figure 6. Bohdanecký plot of wheat β-glucan in 0.5 M NaOH solution.

where M/m is the ratio of the polymer molecular weight to the residue molecular weight (m). Combine eqs 12 and 13

$$[\eta] = \Phi l^3 (C_\infty/m)^{3/2} M^{1/2} = K_\theta M^{1/2} \quad (14)$$

Experiment data were plotted in a form of $[\eta]/M^{1/2}$ versus $M^{1/2}$ in order to obtain K_θ, the value of $[\eta]/M^{1/2}$ in θ conditions (Burchard−Stockmayer−Fixman or BSF plot, Figure 5). This is the simplest extrapolation for estimating unperturbed coil dimensions from perturbed dimensions. The value of K_θ was found to be 0.0020 in 0.5 M NaOH solution. The residue length l was obtained by averaging over β-(1→4) linkages and β-(1→3) linkages with a 7/3 proportion between them by the following equation:[14]

$$l^2 = P_3 l_3^{\,2} + P_4 l_4^{\,2} \quad (15)$$

where P_3 and P_4 are the mole fraction of β-(1→3) linkages and β-(1→4) linkages, and $l_3 = 0.48$ nm and $l_4 = 0.54$ nm are the corresponding residue length, respectively.[38] Using the average molecular weight of residue $m = 162$ and the residue length $l = 0.523$ nm, the characteristic ratio was calculated as 4.97 for samples in 0.5 M NaOH solution. The values of 9.2 and 4.7 of the characteristic ratio for oat β-glucan in water before and after autoclaving were given by Wang et al.[13] The later value was claimed for aggregate-free solution. Gómez et al.[12] reported a characteristic ratio of 7 for barley β-glucan. Theoretical and experimental calculations for barley β-glucan chains by Buliga and Brant[14] yield a result of $C_\infty = 11.7$ and 18, respectively, which were higher than all of the values above from different materials. These results show a wide range of C_∞ values and no correlations between their stiffness and structural features of cereal β-glucans. The discrepancies of C_∞ values are mainly due to the different experimental conditions, which result in the samples with the different degree of aggregation.

3.2.4.2. Wormlike Cylinder Model. The wormlike cylinder model is used for studying the stiffness of semiflexible chains. There are several parameters to describe the dimensions of a polymer chain. The statistical segment length or Kuhn length (l_K) is defined as a length of segment which is sufficiently long so that two adjacent segments could move independently of each other: $l_K = 2l_P$, where l_P is the persistent length. The contour length (L) is the end to end distance when the polymer is pulled to its full extension. In this model, intrinsic viscosity and the characteristic ratio (C_∞) are given by

$$[\eta] = \Phi (l_K/M)^{3/2} M^{1/2} (B_0 + A_0 (l_K/L)^{1/2})^{-3} \quad (16)$$

$$C_\infty = l_K/l \quad (17)$$

The Bohdanecký plot of $(M^2/[\eta])^{1/3}$ vs $M^{1/2}$ is applied for calculation. The slope of the plot is equal to $B_0 \Phi^{-1/3} (M/L l_K)^{1/2}$, and the intercept gives a value for $A_0 \Phi^{-1/3} M/L$. The approximate equations of A_0 and B_0[39] can be used

$$A_0 = 0.46 - 0.53 \log(d/l_K), \text{ and}$$

$$B_0 = 1.00 - 0.0367 \log(d/l_K)$$

where d is the cross-section diameter or the thickness of the molecule.

Figure 6 shows the Bohdanecký plot of wheat β-D-glucan in 0.5 M NaOH solution. The statistical segment length, persistent length, and the characteristic ratio were obtained as 8.83 nm, 4.42 nm, and 16.88, respectively. The value of $C_\infty = 16.88$ means that around 17 glucosyl residues are involved in a statistic segment, which is considered to be rigid. The higher the statistical segment length (or persistent length) and the characteristic ratio, the more rigid the chains will be. Compared to barley and oat β-D-glucans in aqueous solution[12,10] (fractions were obtained by lichenase degradation), the values of persistent length for β-D-glucans follow the order of wheat (4.42 nm) > barley (3.47 nm) > oat (2.30 nm) indicating the same order of the chain stiffness of cereal β-D-glucans. The order of persistent length for cereal β-D-glucans is also coincident with the tri/tetra ratio of cereal β-D-glucans, which has an order of wheat (4.2−4.5) > barley (2.8−3.3) > oat (2.0−2.4).[7] It is believed that the higher tri/tetra ratio leads to higher regularity of polymer chains or higher percentage of the regular segments (consecutive trisaccharide units). The stiffness of wheat β-D-glucan also causes its lower solubility and higher gelation ability. Compared with the random flight model, the influence of the structural features on their chain stiffness is well interpreted by the wormlike cylinder model. Therefore, this model is more suitable for studying the chain stiffness of cereal β-glucans, which is in agreement with Gómez et al.[12] based on barley β-D-glucan and Roubroeks et al.[10] for oat β-D-glucan. The present study implies that wheat β-glucan chain exhibits an extended random coil conformation in solution.

4. Conclusions

Molecular parameters M_w, R_g, R_h, and $[\eta]$ of six fractions of wheat β-glucan were obtained from static and dynamic light scattering and viscometric methods. Critical concentrations (c^*) were predicted by applying the empirical relations between c^* and $[\eta]$. The structure parameter ρ (R_g/R_h) is in the range of 1.52−1.62; the conformation parameter of ν is 0.62. The Mark-Houwink-Sakurada exponents (α) were obtained as 0.78 in 0.5 M NaOH solution. All three structure parameters suggested a random coil conformation of wheat β-glucan in solutions. Two models were used to characterize the chain stiffness. The characteristic ratio $C_\infty = 4.97$ was calculated for samples in 0.5 M NaOH solution using the random flight model. By using the wormlike cylinder model, the statistical segment length (l_K), persistent length, and the characteristic ratio were obtained as

8.83 nm, 4.42 nm, and 16.88, respectively, in 0.5 M NaOH solution, which suggested the extended random coil conformation of wheat β-glucans. The chain stiffness of cereal β-glucans was well interpreted by the wormlike cylinder model. The study of conformation−structure relationship suggested that higher tri/tetra ratio corresponds to a stiffer chain of cereal β-glucans.

Acknowledgment. The authors thank Mr. Ben Huang and Ms. Cathy Wang for technical assistance and Dr. Mei Zhang for valuable suggestions and discussions. The financial support of Heyhoe Mills Ltd. (Ontario, Canada) and OMAF are gratefully acknowledged.

References and Notes

(1) Bhatty, R. S. The potential of hull-less barley. *Cereal Chem.* **1999**, *76*, 589−599.
(2) Klopfenstein, C. F. The role of cereal beta glucans in nutrition and health. *Cereal Foods World* **1988**, *33*, 865−869.
(3) Yokoyama, W. H.; Hudson, C. A.; Knuckles, B. E.; Chiu, M. M.; Sayre, R. N.; Turnlund, J. R.; Schneeman, B. O. Effect of barley β-glucan in durum wheat pasta on human response. *Cereal Chem.* **1997**, *74*, 293−296.
(4) Izydorczyk, M. S.; Biliaderis, C. G. In *Novel Macromolecules in Food Systems*; Doxastakis, G., Kiosseoglou, V., Eds.; Elsevier: Amsterdam, 2000; pp 361−384.
(5) Cui, S. W. *Polysaccharides gums from agricultural products: Processing, structure and functionality*; Technomic Publishing Company: Lancaster, 2001; pp 103−166.
(6) Grimm, A.; Kruger, E.; Burchard, W. Solution properties of β-D-(1→3) (1→4)-glucan isolated from beer. *Carbohydr. Polym.* **1995**, *27*, 205−214.
(7) Cui, W.; Wood, P. J. In *Hydrocolloid*; Nishinari, K., Eds.; Elsevier: Amsterdam, 2000; pp 159−168.
(8) Lazaridou, A.; Biliaderis, C G.; Micha-Screttas, M.; Steele, B. R. A comparative study on structure−function relations of mixed linkage (1→3) linear β-D-glucans. *Food Hydrocolloids* **2004**, *18*, 837−855.
(9) Li, W.; Wang, Q.; Cui, S. W.; Huang, X.; Kakuda, Y. Elimination of aggregates of (1→3) (1→4)-β-D-glucan in dilute solutions for light scattering and size exclusion chromatography study. *Food Hydrocolloids* **2006**, *20*, 361−368.
(10) Roubroeks, J. P.; Mastromauro, D. I.; Andersson, R.; Christensen, B. E.; Åman, P. Molecular weight, structure and shape of oat (1→3) (1→4)-β-D-glucan fractions obtained by enzymatic degradation with lichenase. *Biomacromolecules* **2000**, *1*, 584−591.
(11) Roubroeks, J. P.; Andersson, R.; Mastromauro, D. I.; Christensen, B. E.; Åman, P. Molecular weight, structure and shape of oat (1→3) (1→4)-β-D-glucan fractions obtained by enzymatic degradation with (1→4)-β-D-glucan 4-glucanohydrolase from *Trichoderma reesei*. *Carbohydr. Polym.* **2001**, *46*, 275−285.
(12) Gómez, C.; Navarro, A.; Manzanares, P.; Horta, A.; Carbonell, J. V. Physical and structural properties of barley (1→3) (1→4)-β-D-glucan. Part II. Viscosity, chain stiffness and macromolecular dimensions. *Carbohydr. Polym.* **1997**, *32*, 17−22.
(13) Wang, Q.; Wood, P. J.; Cui, W.; Ross-Murphy, S. B. The effect of autoclaving on the dispersibility and stability of three neutral polysaccharides in dilute aqueous solutions. *Carbohydr. Polym.* **2001**, *45*, 355−362.
(14) Buliga, G. S.; Brant, D. A.; Fincher, G. B. The sequence statistics and solution configuration of a barley (1→3) (1→4)-β-D-glucan. *Carbohydr. Res.* **1986**, *157*, 139−156.
(15) Cui, W.; Wood, P. J.; Blackwell, B.; Nikiforuk, J. Physicochemical properties and structural characterization by two-dimensional NMR spectroscopy of wheat β-D-glucan−comparison with other cereal β-D-glucans. *Carbohydr. Polym.* **2000**, *41*, 249−258.
(16) Wang, Q.; Wood, P. J.; Huang, X.; Cui, W. Preparation and characterization of molecular weight standards of low polydispersity from oat and barley (1→3) (1→4)-β-D-glucan. *Food Hydrocolloids* **2003**, *17*, 845−853.
(17) Vårum, K. M.; Smidsrød, O.; Brant, D. A. Light scattering reveals micelle-like aggregation in the (1→3),(1→4)-β-D-glucans from oat aleurone. *Food Hydrocolloids* **1992**, *5*, 497−511.
(18) Li, W.; Cui, S. W.; Kakuda, Y. Extraction, fractionation, structural and physical characterization of wheat β-D-glucans. *Carbohydr. Polym.* **2005**, In press.
(19) Kratochvil, P. *Classical light scattering from polymer solutions*; Elsevier: Amsterdam, 1987; pp 17−22.
(20) Burchard, W. In *Physical Techniques for the Study of Food Biopolymers*; Ross-Murphy, S. B., Ed.; Blackie Academic and Professional: Glasgow, 1994; pp 151−213.
(21) Stock, R. S.; Ray, W. H. Interpretation of photon correlation spectroscopy data: A comparison of analysis methods. *J. Polym. Sci., Part B: Polym. Phys.* **1985**, *23*, 1393−1447.
(22) Vårum, K. M.; Smidsrød, O. Partial physical and chemical characterisation of (1→3) (1→4)-β-D-glucans from oat (*Avena sativa* L.) aleurone. *Carbohydr. Polym.* **1988**, *9*, 103−117.
(23) Doublier, J. L.; Wood, P. J. Rheological properties of aqueous solutions of (1→3) (1→4)-β-D-glucan from oats (*Avena sativa* L.). *Cereal Chem.* **1995**, *72*, 335−340.
(24) Gómez, C.; Navarro, A.; Manzanares, P.; Horta, A.; Carbonell, J. V. Physical and structural properties of barley (1→3) (1→4)-β-D-glucan. Part I. Determination of molecular weight and macromolecular radius by light scattering. *Carbohydr. Polym.* **1997**, *32*, 7−15.
(25) Izydorczyk, M. S.; Biliaderis, C. G.; Macri, L. J.; MacGregor, A. W. Fractionation of oat (1→3) (1→4)-β-D-glucans and characterisation of the fractions. *J. Cereal Sci.* **1998**, *27*, 321−325.
(26) Tanford, C. Physical *chemistry of macromolecules*; Wiley: New York, 1961.
(27) Vårum, K. M.; Martinsen, A.; Smidsrød, O. Fractionation and viscometric characterisation of a (1→3) (1→4)-β-D-glucan from oat and universal calibration of a high performance size-exclusion chromatographic system by use of fractionated β-glucans, alginates and pullulans. *Food Hydrocolloids* **1991**, *5*, 363−374.
(28) Reese, E. T.; Perlin, A. S. Enzymic preparation of 3-*O*-β-cellobiosyl D-glucose. *Biochem. Biophys. Res. Commun.* **1963**, *12*, 194−197.
(29) Morris, E. R. In *Frontiers in Carbohydrate Research I: Food Application*; Millane, R. P., BeMiller, J. N., Chandrasekaran, R., Eds; Elsevier: New York, 1989; pp 132−163.
(30) Robinson, G.; Ross-Murphy S. B.; Morris, E. R. Viscosity-molecular weight relationships, intrinsic chain flexibility, and dynamic solution properties of guar galactomannan. *Carbohydr. Res.* **1982**, *107*, 17−32.
(31) Burkus, Z.; Temelli, F. Determination of the molecular weight of barley β-glucan using intrinsic viscosity measurements. *Carbohydr. Polym.* **2003**, *54*, 51−57.
(32) Vaikousi, H.; Biliaderis, C. G.; Izydorczyk, M. S. Solution flow behavior and gelling properties of water-soluble barley (1→3) (1→4)-β-glucans varying in molecular size. *J. Cereal Sci.* **2004**, *39*, 119−137.
(33) Lazaridou, A.; Biliaderis, C. G.; Izydorczyk, M. S. Molecular size effects on rheological properties of oat β-glucans in solutions and gels. *Food Hydrocolloids* **2003**, *17*, 693−712.
(34) Cuvelier, G.; Launay, B. Concentration regimes in xanthan gum solutions deduced from flow and viscosity properties. *Carbohydr. Polym.* **1986**, *6*, 321−333.
(35) Kirkwood, J. G.; Riseman, J. The intrinsic viscosities and diffusion constants of flexible macromolecules in solution. *J. Chem. Phys.* **1948**, *16*, 565−573.
(36) Teraoka, I. *Polymer solutions: An introduction to physical properties*; John Wiley & Sons: New York, 2001; pp 209−221.
(37) Böhm, N.; Kulicke, W. M. Rheological studies of barley (1→3) (1→4)-β-glucan in concentrated solution: Mechanistic and kinetic investigation of the gel formation. *Carbohydr. Res.* **1999**, *315*, 302−311.
(38) Morris, E. R.; Ross-Murphy, S. B. *Tech. Carbohydr. Metabolism B* **1981**, *310*, 1−46.
(39) Bohdanecký, M. New method for estimating the parameters of the worklike chain model from the intrinsic viscosity of stiff-chain polymers. *Macromolecules* **1983**, *16*, 1483−1492.

BM050625V