Rheological Properties of Aqueous Solutions of (1→3)(1→4)-β-D-Glucan from Oats (*Avena sativa* L.)[1]

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Cereal Chem. 72(4):335–340

ABSTRACT

The rheological behavior of partially hydrolyzed oat (1→3)(1→4)-β-D-glucan was compared to that of the original unhydrolyzed β-D-glucan. Flow and oscillatory shear measurements of the nonhydrolyzed sample in aqueous solution showed behavior typical of noninteracting polysaccharides, such as guar gum, in solution. The rheology of partially hydrolyzed samples shifted toward the more gel-like behavior observed with polymers, which tend to aggregate and form a three-dimensional macromolecular network. The aggregation may arise from a tendency of the β-glucan to self-associate through cellulose-like sequences in the structure. However, the reason why this behavior was not observed with unhydrolyzed oat gum is not understood. It is unlikely that structural variations, such as the amount of cellulose-like sequences, accounted for the different rheology, because quantitative analysis of oligosaccharides released by lichenase evidenced only minor differences between intact oat β-glucan and hydrolyzed samples. A more likely explanation is that because of their lower molecular weight, the partially hydrolyzed molecules are more mobile and diffuse more easily. Hence, they have a greater probability of forming aggregates.

The viscosity of barley (1→3)(1→4)-β-D-glucan (β-glucan) interferes with the brewing process (Bamforth 1985) and limits the value of feed barley for poultry (Campbell and Bedford 1992). However, because of the high viscosity of cereal β-glucan solutions, it was suggested that oat gum (70–80% β-glucan) could have commercial value as a thickening agent in food formulations (Wood 1984; Autio et al 1987, 1992). Furthermore, the physiological response to consumption of oat β-glucan may be viscosity-dependent. Indeed, postprandial blood glucose and insulin rise is inversely related to log[viscosity] (Wood et al 1994a).

Despite this evident importance of viscosity, there have been few reports on the rheology of these polysaccharides, although flow properties have been described using coaxial cylinder viscometry (Wood 1984; Autio et al 1987, 1992). Oat gums showed non-Newtonian shear-thinning behavior above ~0.2% concentration. In these reports, flow curves were described using the power law equation:

$$\sigma = k \times \dot{\gamma}^n$$

or

$$\eta_a = k \times \dot{\gamma}^{n-1}$$

where σ = the shear stress; $\dot{\gamma}$ = the shear rate; and η_a = the apparent viscosity at shear rate $\dot{\gamma}$. The power law parameters k and n were compared to data for guar gum. It must be emphasized that the power law relationship has a limited shear-rate range of validity; measurements generally should be limited to, at most, two decades of shear rate. However, a comparison of data in terms of the power law parameters is valid, provided the same shear-rate range is explored (Wood 1993). The main conclusion that can be drawn from the results of Autio et al (1987, 1992) and Wood (1984, 1993) is that oat gum in solution, and hence β-glucan, is highly shear-thinning and not thixotropic. It is comparable with other polysaccharides, galactomannans for instance, with respect to thickening properties.

An improved understanding of the rheology of β-glucan requires the flow properties to be measured over a wide range of shear rates, as reported extensively for other hydrocolloids (Morris et al 1981, Launay et al 1986). Such measurements provide the limiting, or zero shear rate, Newtonian viscosity (η_0) that is related to molecular weight and concentration (Robinson et al 1982, Launay et al 1986). In addition, the viscoelastic behavior in dynamic conditions (in oscillatory shear) should be examined. Variations of the storage modulus (G') and of the loss modulus (G'') as a function of frequency (ω) provide information on the organization of the macromolecules in the medium (Clark and Ross-Murphy 1987).

The aim of the present work was to investigate the rheology of native and partially acid-hydrolyzed oat gums used in clinical studies of postprandial blood glucose and insulin levels (Wood et al 1994a). Flow and viscoelastic measurements were used and differences between hydrolyzed and unhydrolyzed samples were evaluated in structural terms.

MATERIALS AND METHODS

Oat and Guar Gums

Oat gum (OG0) was prepared in the POS Pilot Plant, Saskatoon, SK, as previously described (Wood et al 1989).

Acid-hydrolyzed oat gum was also prepared at the POS Pilot Plant. OG (100 g) was slurried in ethanol (95%, 400 ml) and the suspension was added gradually to 5L of water in a Waring Blendor and mixed. The dispersed gum was transferred to a 20-L, Teflon-coated vessel fitted with an overhead stirrer and stirred with a Teflon-coated paddle as it was heated to 70°C. Hydrochloric acid (0.2*M*, 5L) was prepared in a glass vessel and preheated to 70°C. It was added to the mixture and then stirred at 70°C for 15 min (OG15) or 60 min (OG60). The mixtures were rapidly cooled in an ice bath to ~30°C. The pH was adjusted to 6.5–7.0 with 1*M* NaOH, followed by 0.1*M* NaOH. An equal volume of ethanol (95%) was slowly added to the hydrolyzed gum solutions with vigorous stirring, and after settling, the precipitates were recovered by siphoning and centrifugation. The precipitates were washed with 47.5% ethanol in the Waring Blendor, recovered, and redispersed in 95% ethanol, then filtered and dried in an oven at 30°C. Yields of OG15 and OG60 were 87 and 83%, respectively.

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The guar gum sample that was characterized for comparison was a high-purity gum (protein content < 0.2%) obtained from Meyhall Chemical (Switzerland).

Preparation of Solutions

Solutions were prepared by sprinkling dry sample into water while stirring rapidly, heating at 60°C for 30 min, and filtering. Concentrations were estimated by drying an aliquot of the solution overnight at 102°C. Concentrations are reported as β-glucan content (g/100 ml, %w/v) based on the β-glucan content of oat gums (~80–89%) (Table I).

Rheological Measurements

Two types of viscometers were used for flow behavior measurements, depending on the viscosity range. For concentrations that are low and intermediate, a highly sensitive coaxial cylinders viscometer (Low-Shear 40, Mettler, Zürich, Switzerland) (6 mm i.d.; 6.5 mm o.d.; 18 mm height) was used. This high sensitivity viscometer also allowed measurements to be performed at very low concentrations to determine intrinsic viscosity. For the highest concentrations, the Rheometrics fluid spectrometer (RFS II, Rheometrics Inc., Piscataway, NJ) was used with a cone-plate attachment (5 cm diameter; 2° cone angle). All measurements were performed at 25°C.

Viscoelastic measurements were made in dynamic conditions (oscillatory shear) using the RFS II in the frequency range 10^{-2} to 10^{2} rad/sec. Amplitude of shear deformation (30%) was verified to be within the limits of linear viscoelasticity.

Molecular Weight Estimation of Oat β-Glucan

Molecular weights of oat β-glucan were estimated from the chromatographic peak using a high-performance size-exclusion chromatography (HPSEC) column (TSK 60XL, Bio-Rad Laboratories, Mississauga, ON) calibrated with β-glucan standards as described by Wood et al (1991).

Lichenase Treatment and Analysis of Oligosaccharides

(1→3)(1→4)-β-D-Glucan-4-glucanohydrolase (EC 3.2.1.73, lichenase) was part of a β-glucan analysis kit (supplied by Biocon, Lexington, KY, now also available from Megazyme, Sydney, NSW, Australia). One unit of enzyme is the amount of enzyme that releases 1 μmol/min of reducing sugar, measured as glucose equivalents, from an oat β-glucan substrate.

Typically, 10 mg of oat β-glucan was dissolved in 5 ml of 20 m*M* sodium phosphate buffer in glass screw-capped tubes and lichenase solution (8 units); phosphate buffer was added to a total volume of 10 ml. The tubes were capped and incubated with stirring at 50°C for 90 min. Aliquots of the digest were diluted with water 20-fold for analysis of the tri- and tetrasaccharide products, but the solution was injected undiluted for determination of the higher degree of polymerization (DP) oligosaccharides. A Dionex system (Dionex, Sunnyvale, CA) with a Carbopac PA1 column (4 × 250 mm) and pulsed amperometric detection (PAD) with a gold electrode was used to analyze the oligosaccharides. Samples were filtered (0.45 μm) before analysis. For separation of tri- to nonasaccharides, eluent A was 150 m*M* sodium acetate in 150 m*M* sodium hydroxide, and eluent B was 150 m*M* sodium hydroxide. Elution was with 70% eluent A and 30% eluent B for 1 min, then 100% eluent A for 9 min, which was continued for 1 min. The initial conditions were maintained for 5 min between each injection of sample., The flow rate (ambient temperature) was 1.0 ml/min. Pulse potentials (E = volts) and durations (t = ms) were: $E_1 = 0.1$, $t_1 = 300$; $E_2 = 0.6$, $t_2 = 120$; $E_3 = -0.8$, $t_3 = 300$. Response time of the detector was 3.0 sec. The instrument was controlled and data processed using Dionex AI 450 software.

Isolation of Insoluble Precipitate from Lichenase-Treated Oat β-Glucan

Oat gum or hydrolyzed oat gum (2 g) was dissolved in 0.05*M* phosphate buffer (600 ml, pH 6.9) containing 10 m*M* NaCl by heating (60°C) and stirring for 3 hr. Insoluble material was removed by centrifugation (33,000 × *g*, 30 min). The supernatant was treated with 30 units of hog pancreatic α-amylase (Type 1A, DFP-treated, Sigma) for 1 hr at room temperature. The solution was heated (70–75°C) for 45 min and centrifuged (33,000 × *g*, 1 hr). Then 2-propanol (600 ml) was added dropwise to the supernatant. The precipitate was collected by centrifugation (4,100 × *g*), redispersed (using a Virtis homogenizer) and recentrifuged in 50% 2-propanol, dispersed in 100% 2-propanol, filtered, and air-dried. The α–amylase treated gum was wetted with ethanol (8 ml), dissolved in 20 m*M* phosphate buffer (200 ml, pH 6.5) and treated with lichenase (150 units) for 4 hr at 40°C. The solution was heated to 80°C for 15 min and stored at 5°C overnight. The precipitate was recovered by centrifugation (33,000 × *g*, 30 min) and washed twice with water, suspended in 2-propanol, and recovered on a sintered glass filter.

RESULTS

Partial acid hydrolysis significantly reduced the weight-average molecular weight (M_W) and intrinsic viscosity ([η]) and somewhat increased the purity of oat gum (Table I). The M_W of guar gum was estimated from [η] using the Mark-Houwink coefficients ($[\eta] = 3.8\ 10^{-4}\ M_W^{\ 0.723}$) determined by Robinson et al (1982). The M_W and [η] of the guar gum sample used are close to those of the unhydrolyzed oat gum, suggesting that the overall conformation of the two polysaccharides is comparable. It should be noted that the number of data points (3) in Table I are insufficient to obtain reliable Mark-Houwink coefficients for oat β-glucan. Moreover, this equation is valid provided each sample of the polysaccharide adopts a similar conformation, which may not be the case for lower molecular weight oat gums.

Flow Behavior of Unhydrolyzed Oat Gum

The behavior of solutions of oat gum below ~0.3% was essentially Newtonian (not shown), but as concentration was increased beyond this value, solutions of OG0 developed non-Newtonian shear-thinning or *pseudoplastic* behavior (Fig. 1). Above a minimum shear rate, the apparent viscosity decreased as the shear rate increased and, as usual for polysaccharide solutions, this shear-thinning behavior was more pronounced as the concentration increased. No thixotropy (time dependency) was evident. A

TABLE I
Analytical Characterisitics of Untreated (OG0) and Acid-Hydrolyzed (OG15 and OG60) Oat Gums and Guar Gum

Sample	$M_w \times 10^{-3}$	[η] dl/g	Composition				
			β-Glucans	Starch	Pentosan	Protein	Ash
OG0	1,200	9.63	81	2.0	3.4	5.2	1.8
OG15	360	3.90	89	1.6	Tr[a]	3.7	0.5
OG60	100	2.58	89	1.5	Tr	3.4	0.7
Guar gum	1,300[b]	11.1	...	...	...	Tr	Tr

[a] Trace.
[b] From [η] using Mark-Houwink equation (Robinson et al 1982).

limiting Newtonian viscosity (η_0) was reached in the low shear rate range. The η_0 increased from ~0.08 to ~1.2 Pa·s as the concentration increased from 0.39 to 0.78%. This illustrates the dramatic concentration dependence of η_0. Autio (1988 and personal communication 1993) also found strong variation of η_0 with concentration of oat gum, but the reported η_0 values for corresponding concentrations were higher. The molecular weight of the sample used by Autio (1988) was estimated at 2×10^6 to be compared with 1.3×10^6 for the β-glucan in this study, suggesting that the different values for η_0 reflect the difference in molecular weights.

Viscoelastic Behavior of Unhydrolyzed Oat Gum

The mechanical spectra (variations of G' and G'' as a function of frequency) of two concentrations of OG0 (0.39 and 0.78%) showed that, at low frequency, G'' was higher than G', and that both parameters varied sharply with frequency: $G'' \alpha \omega$ and $G' \alpha \omega^2$ (Fig. 2). This behavior is liquid-like. As frequency increased, because G' increased faster than G'', a cross-over of the curves tended to take place beyond which $G' > G''$. This was particularly clear at the highest concentration. The response of the material beyond the cross-over frequency was more solid-like. Such viscoelastic behavior is typical of macromolecular solutions, whatever the chemical nature of the molecule, whether synthetic polymer or polysaccharide (Doublier et al 1993).

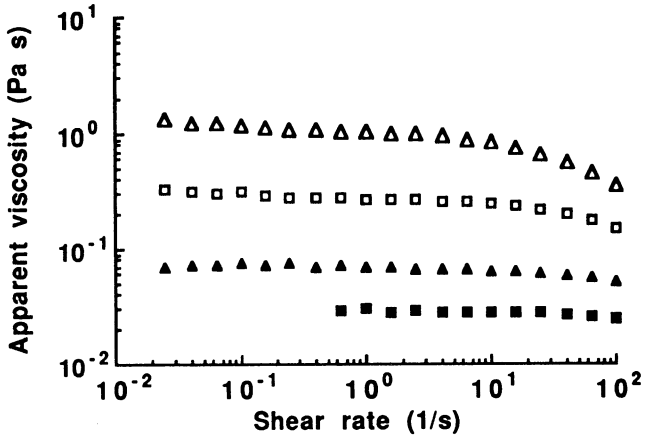

Fig. 1. Apparent viscosity as a function of shear rate of unhydrolyzed oat gum (OG0) solutions. Concentrations: 0.78% (Δ), 0.58% (□), 0.39% (▲), 0.29% (■).

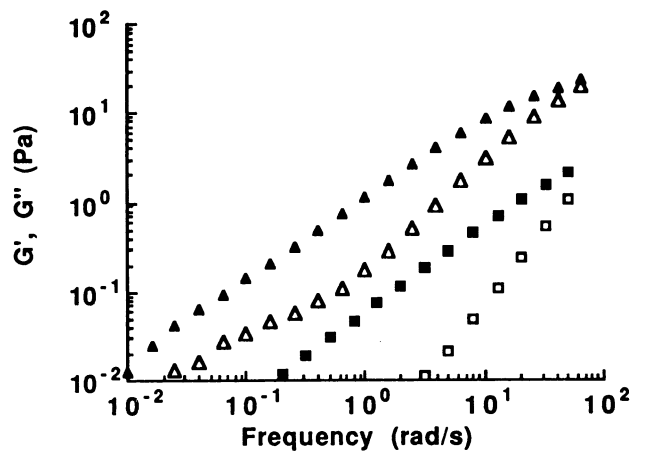

Fig. 2. Storage modulus (G') and loss modulus (G'') as a function of angular frequency of unhydrolyzed oat gum (OG0) solutions. Concentrations: 0.78% (Δ = G', ▲ = G''), 0.39% (□ = G', ■ = G'').

Flow Behavior of Hydrolyzed Samples

Flow curves of OG15 for concentrations from 0.55 to 2.75% show typical shear-thinning behavior (Fig. 3). The apparent viscosity at relatively high shear rate, 100 sec^{-1} for instance, was much lower than for the unhydrolyzed sample OG0. This was an expected consequence of depolymerization of the β-glucan. However, at low shear rates, OG15 behaved in a totally different way from the unhydrolyzed β-glucans; the apparent viscosity continued to increase as the shear rate decreased. These tendencies were more pronounced at lower concentrations.

The flow curves of OG60 for concentrations from 1.15 to 5.5% show the same trends observed with OG15 (Fig. 4). The apparent viscosity was considerably lower than those of OG0 and OG15, reflecting the greater extent of depolymerization; the chromatographic peak MW was 12-fold lower than for OG0 and fourfold lower than OG15. At low shear rates (OG15), the apparent viscosity continued to increase as the shear rate decreased, and this phenomenon was even more pronounced at lower concentration.

Viscoelastic Behavior of Hydrolyzed Samples

The unusual behavior of OG15 was confirmed by the mechanical spectra of solutions (1.37 and 2.75%) at low frequency where a cross-over of the $G'(\omega)$ and $G''(\omega)$ curves was evident (Fig. 5);

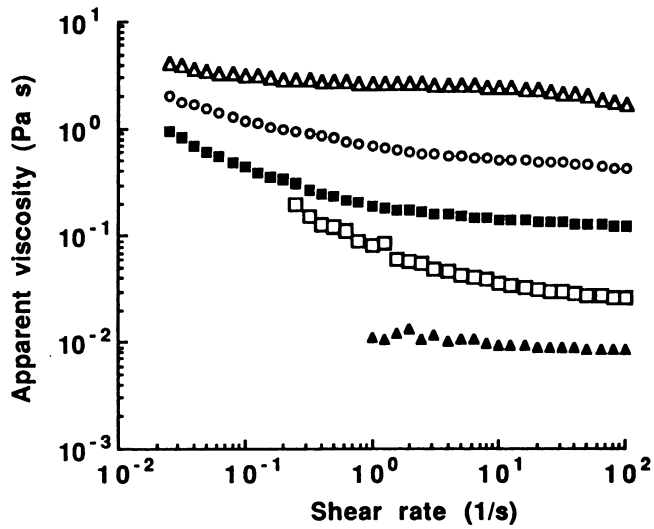

Fig. 3. Apparent viscosity as a function of shear rate of acid-hydrolyzed oat gum (OG15) solutions. Concentrations: 2.75% (Δ), 1.90% (○), 1.37% (■), 0.82% (□), 0.55% (▲).

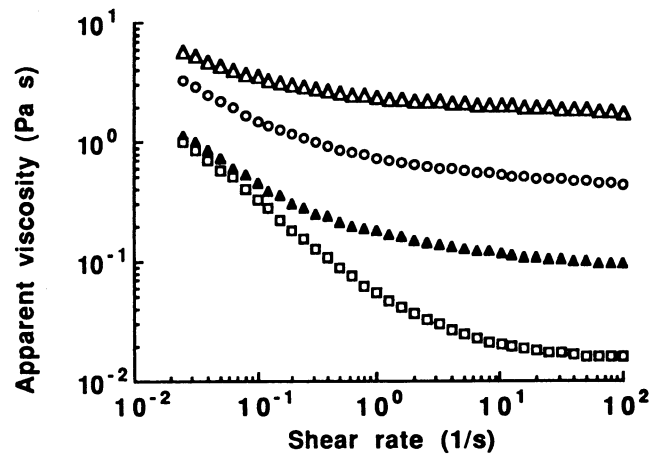

Fig. 4. Apparent viscosity as a function of shear rate of acid-hydrolyzed oat gum (OG60) solutions. Concentrations: 5.5% (Δ), 3.90% (○), 2.75% (▲), 1.15% (□).

G' became higher than G'' at low frequency. This occurred at ~0.01 and 1 rad/sec, for the 2.75 and 1.37% solutions, respectively. These cross-overs resulted from a tendency of the $G'(\omega)$ curve to level towards the horizontal. At higher frequency, on the other hand, OG15 behaved similarly to OG0; the loss modulus G'' and the storage modulus G' increased as the frequency increased and the frequency dependence of G' was stronger than that of G''. However, the G'-G'' crossover was not seen, presumably because it occurred beyond 100 rad/sec, the highest frequency accessed in these experiments. Thus, at high frequency, the viscoelastic behavior of OG15 was similar to that of the unhydrolyzed sample (OG0), but at low frequency, $G'(\omega)$ remained almost constant, indicating that the system was behaving as a gel as a result of intermolecular interactions. However, the low values of G' at the plateau (<0.1 Pa) indicated that the cross-linked network was extremely tenuous.

Similar tendencies were exhibited by OG60 (Fig. 6, concentration 2.75%). At low frequencies, the G' tended to level-off to a plateau value that was higher than the plateau value found with OG15 at the same concentration (<0.1 Pa). Also, the G'-G'' crossover took place at a higher frequency than for OG15 at the same concentration. Viscoelastic data did, therefore, confirm the conclusions of the flow measurements: the rheological characteristics of oat β-glucan were changed by depolymerization, and the changes were more pronounced for lower molecular weight β-glucan.

Oligosaccharides Released by Lichenase

High-performance anion-exchange chromatography (HPAEC) with pulsed amperometric detection (PAD) (Wood et al 1994b) was used to analyze the soluble oligosaccharides released by lichenase from the β-glucans. The data, summarized in Table II, showed no significant structural differences between OG0, OG15, and OG60.

The carbohydrate portion, from an insoluble residue also formed during lichenase treatment of oat β-glucan, is composed predominantly of material from DP 9-15 (Wood et al 1994b). Accurate analysis of this material is difficult, but essentially, the yields from the two acid-hydrolyzed gums (3.3% of β-glucan content for OG15 and 3.6% for OG60) were similar and not greatly different from those of unhydrolyzed gum (4.9%).

DISCUSSION

The rheological behavior of unhydrolyzed oat gum in solution resembles that of other nongelling polysaccharides often used as food thickening agents, such as galactomannans (guar gum, locust bean gum).

The overall shape of the flow curves was typical of macromolecular solutions with topological entanglements (Ferry 1980). The properties are known to be directly related to the molecular weight of the polymers and to depend strongly upon concentration (Morris et al 1981, Robinson et al 1982, Launay et al 1986). A convenient parameter used to characterize these solutions is the reduced concentration, defined as the product of the concentration and the intrinsic viscosity: $c[\eta]$.

Variations of the specific viscosity at zero shear rate

$$\eta_{spo} = (\eta_0 - \eta_{solvent})/\eta_{solvent}$$

plotted as a function of the reduced concentration (Fig. 7) for OG0 and the guar gum sample used for comparison, show similar curves for the two samples, despite differences in their chemical structure and molecular weight. The curve can be divided into

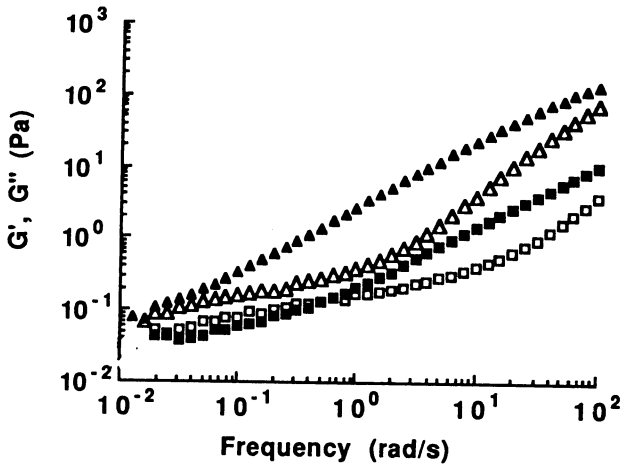

Fig. 5. Storage modulus (G') and loss modulus (G'') as a function of angular frequency of acid-hydrolyzed oat gum (OG15) solutions. Concentrations: 2.75% (Δ = G', ▲ = G''), 1.37% (□ = G', ■ = G'').

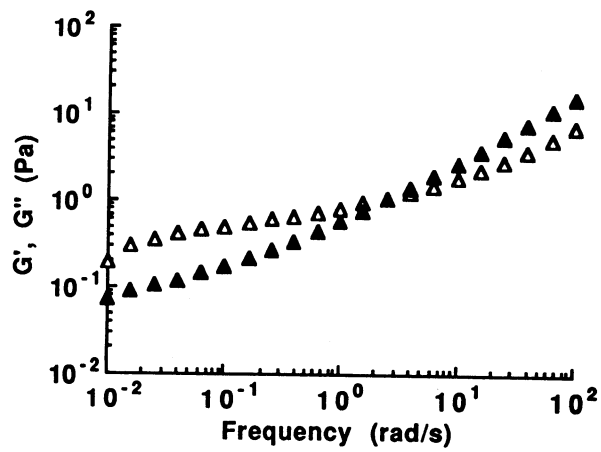

Fig. 6. Storage modulus (G') and loss modulus (G'') as a function of angular frequency of acid-hydrolyzed oat gum (OG60) solutions. Concentration: 2.75% (Δ = G', ▲ = G'').

TABLE II
Analysis of Oligosaccharides from Lichenase Digest of Untreated (OG0) and Acid-Hydrolyzed (OG15 and OG60) Oat Gum

Sample	Nomalized Area, %								
	3[a]	4	5	6	7	8	9	3+4	5–9
OG0	58.09	34.18	3.03	2.22	0.37	0.68	1.43	92.3	7.7
	(0.16)	(0.09)	(0.09)	(0.06)	(0.02)	(0.08)	(0.08)		
OG15	58.87	33.70	3.04	2.12	0.33	0.66	1.28	92.6	7.4
	(0.26)	(0.10)	(0.12)	(0.06)	(0.02)	(0.03)	(0.05)		
OG60	58.36	33.55	3.34	2.29	0.37	0.71	1.39	91.9	8.1
	(0.02)	(0.13)	(0.05)	(0.04)	(0.01)	(0.02)	(0.03)		

[a] Degree of polymerization.

three straight lines, with the following slopes, defining three concentration regimes:

$$c[\eta] < 0.7, \text{ slope} = 1.1$$
$$0.7 > c[\eta] < 2.5, \text{ slope} = 1.8$$
$$c[\eta] > 2.5, \text{ slope} = 3.9$$

These values are in agreement with those reported in the literature for most random coil polysaccharides (Launay et al 1986). Of particular interest is the first critical concentration ($c[\eta] = 0.7$), which corresponds to the entanglement concentration. The value we found here is very close to that predicted theoretically for macromolecular solutions (Graessley 1974). This sharp concentration dependence explains why slight variations in concentration or in molecular weight (i.e., [η]) can result in significant differences in η_o. For example, the value for η_o for OG0 at 0.78% was ~1.2 Pa·s, whereas Autio (1988 and personal communication 1993) reported η_o was ~1.8 Pa·s at 0.44%. The molecular weight of Autio's sample was estimated at 2×10^6 compared to 1.3×10^6 for OG0. Because the specific limiting viscosity η_{spo} depends strongly upon [η], and hence the molecular weight, slight variations in these parameters yield dramatic variations in η_{spo} and η_o. The same type of plot cannot be obtained with OG15 and OG60 because, in that case, there is no limiting Newtonian viscosity at low shear rate.

Viscoelastic behavior of unhydrolyzed β-glucan is comparable to that exhibited by guar gum or locust bean gum (Robinson et al 1982, Richardson and Ross-Murphy 1987, Doublier et al 1993) and can be interpreted in a similar way. The terminal zone (at low frequency) and the beginning of the plateau zone (at high frequency) of the complete mechanical spectrum were observed within the frequency range explored. Thus, the rheology of solutions of unhydrolyzed β-glucan, like that of galactomannans, was mainly governed by the degree of entanglement of individual polymer chains. There was no evidence of specific macromolecular interactions in these systems. The behavior is primarily controlled by the molecular dimensions of the β-glucan (molecular weight, intrinsic viscosity).

The behavior of hydrolyzed samples was unforeseen. The main anticipated effect was a decrease of the apparent viscosity due to depolymerization, with the overall behavior remaining that of a macromolecular solution. In fact, the properties of the hydrolyzed samples remained similar to those of unhydrolyzed oat gums at the higher shear rates and frequencies examined, but differed at low shear rates and at low frequency. The similarities mean that a large part of the behavior is comparable to that of unhydrolyzed oat gum or guar gum and is governed by molecular entanglements. The deviations at low shear rate or low frequency require more detailed discussion. On one hand, an increase in apparent viscosity at low shear rate as shear rate decreases is indicative of the existence of a yield stress, classically ascribed to the presence of particles in the macromolecular medium, but it also can be related to weak intermolecular interactions. On the other hand, viscoelastic data at low frequency indicate that there is a tendency for the system to display gel-like properties. This may arise from intermolecular interactions forming physical cross-links. Such processes represent an aggregation phenomena. To support this assertion, two hypotheses can be proposed.

The first is related to the presence of proteins. Similar overall rheological behavior in both flow and viscoelastic properties has been observed in polysaccharide-bovine serum albumin mixtures (Castelain et al 1986) and was ascribed to the concentration and aggregation of proteins through a phase-separation phenomenon. The result was a suspension of particles, the protein aggregates, dispersed in a macromolecular medium. Because the oat gums contain some protein (Table I), this may provide an explanation for the observed phenomena. This process would be governed by the protein content, and consequently, the higher the protein concentration, the more dramatic the effect. However, because the partially hydrolyzed gums contained less protein than did the unhydrolyzed gum, this mechanism appears unlikely, although it is not entirely ruled out.

The second hypothesis seems more plausible and is the consequence of self-aggregation of the β-glucan. This tendency was described by Vårum et al (1992) for β-glucans samples that had been sonicated and had relatively low molecular weights, with intrinsic viscosities ranging from 1.10 to 3.5 dl/g, values on the same order as those of the hydrolyzed samples in the present work. From light-scattering experiments and theoretical calculations, these authors suggested that a proportion of the macromolecules (~10%) would be involved in the formation of large, cooperatively stabilized clusters. The present rheological data may be interpreted by assuming that hydrolyzed samples of β-glucan tend to aggregate, while unhydrolyzed β-glucan does not (in the time-scale considered here). This aggregation implies that an amount of the polymer chains are involved in intermolecular interactions which might give either a suspension or a network. The two processes differ only in the details, both arising from the same basic mechanism of aggregation of macromolecular chains. In the case of a suspension, the volume fraction of the dispersed particles would remain relatively low. In the case of a network, the proportion of cross-links would be too small to yield a true gel. Therefore, in both cases, the properties reflect the superposition of two behaviors. The major one is governed by the macromolecular entanglements. The second one arises from aggregation of the macromolecules and intermolecular interactions in excess of chain entanglements, and can lead either to a suspension or a weak network.

A plausible foci for aggregation in (1→3)(1→4)-β-D-glucan chains would be cellulose-like sequences of more than three contiguous β-(1→4)-linked glucose units (Fincher and Stone 1986). The enzyme lichenase, a (1→3)(1→4)-β-D-glucan-4-glucanohydrolase, specifically cleaves the (1→4)-glycosidic bond of 3-substituted glucose residues in the β-glucans. The major products are 3-O-β-cellobiosyl-D-glucose and 3-O-β-cellotriosyl- D-glucose (Table II), but cellodextrin-like oligosaccharides of higher DP are produced from regions of the polymer containing more than three consecutive 4-linked glucose residues. The tendency of such regions to aggregate was evident during digestion of oat (or barley) β-glucan with the lichenase, when a precipitate was formed (Woodward et al 1983, Wood et al 1991). This material, rendered

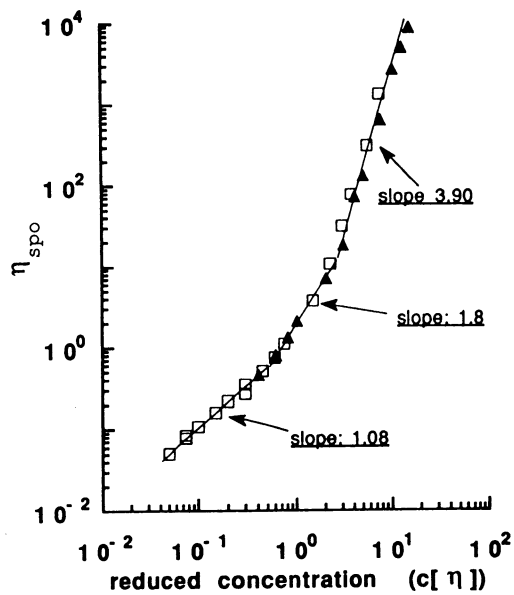

Fig. 7. Limiting specific viscosity (η_{spo}) as a function of the reduced concentration (c[η]). OG0 (□), guar gum (▲).

insoluble by lichenase, was composed of cellulose-like oligosaccharides terminated on their reducing end by β–(1→3)-linked glucose.

After lichenase treatment, ~10% (by weight) of the β-glucans were analyzed as soluble oligosaccharides with DP ≥ 5 (Table II). Of the lichenase-released oligosaccharides with DP > 5, the predominant product was DP 9, arising from sequences of nine consecutive (1→4)-linked glucose units in the intact polysaccharide (Wood et al 1994b). This and higher DP oligosaccharides were the major components of the material made insoluble by lichenase and, like higher DP cellodextrins and cellulose itself, were essentially water-insoluble. The cellulose-like structural features appear to be conserved in oat and barley β-glucan (Wood et al 1994b) and clearly serve some useful purpose to the plant, such as cell-wall cohesion.

Because partial acid hydrolysis might have cleaved glycosidic bonds preferentially within different structural regions of the β-glucan, it was possible that the different behavior of hydrolyzed β-glucan was structural in origin. However, the present investigations were unable to identify changes in average structure brought about by the acid treatment (Table II).

A plausible explanation for the rheological behavior of OG15 and OG60 might be that the hydrolyzed macromolecules are more mobile because of lower molecular weight. The molecules would diffuse more readily and, hence, have a greater probability of achieving the proximity of structural regions required for aggregation. It seems likely that the cellulose-like zones in the molecule would be involved in this aggregation, but regions of structural regularity might also perform this role. The aggregates, or clusters, would act as physical cross-links between β-D-glucan molecules and, hence, would yield a tenuous network in excess of chain entanglements.

ACKNOWLEDGMENTS

We thank J. Weisz for assistance in structural analysis by HPAEC of the oligosaccharides released by lichenase. We also are indebted to G. Llamas for her skillful technical assistance.

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[Received May 25, 1994. Accepted December 19, 1994.]