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Microwave assisted dissolution of β-glucan in water — implications for the characterisation of this polymer

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Abstract

Microwave heating in a high-pressure vessel was used to assist the dissolution of a high molecular weight β-glucan in water. These samples could not be fully dispersed by heating and stirring at 80°C. This was evident from the detection of large aggregates by light scattering and from low sample recovery after filtration. When the pre-hydrated solution was heated in a microwave at a temperature lower than 90°C, aggregation could not be effectively reduced within 30 min. However, when the solutions were heated to 100, 110 and 121°C and held at these temperatures for 4–10 min, good dispersability was achieved without degradation. Sample recovery increased from 75 ± 5% to 98 ± 2%. Longer time at high temperatures (110 and 121°C) induced polymer degradation. Crown Copyright © 2002 Published by Elsevier Science Ltd. All rights reserved.

Keywords: Microwave heating; Aggregates; β-glucan solution

1. Introduction

In our laboratory we have observed that some purified, high molecular weight β-glucan samples do not dissolve properly after the usual treatment of stirring at 80°C for 0.5–1 h, followed by 60°C for 3 h (or sometimes overnight). This was revealed by the detection of large aggregates by light scattering (LS), and from molecular weight measurements. The results were time dependent and showed large variations. Taking a longer time to dissolve the sample is time consuming and can lead to polymer degradation. Excessive heat and pressure treatment may also lead to polymer degradation. A previous study showed that autoclaving at 121°C for 15 min caused severe polymer degradation (Wang, Wood, Cui & Ross-Murphy, 2001), although it significantly reduced polymer aggregation. One problem in autoclaving is that the heating and cooling cycle is relatively long. In our case, it took about 15–20 min to reach 121°C and 20–30 min to cool down to 90°C. This made it difficult to control the precise time of treatment. Microwave heating used in this experiment allowed the sample to be heated to a high temperature and cooled down again in a short period. The purpose of this study was to explore the possibility of using microwave heating in a high-pressure vessel to prepare fully dispersed, high molecular weight β-glucan solutions without depolymerisation of the β-glucan.

2. Materials and methods

Oat β-glucan was extracted and purified as described previously (Wood, Weisz & Blackwell, 1991). β-glucan solutions were prepared by first dissolving the weighed sample at 80°C for 0.5–1 h, followed by 60°C for 3 h at a concentration of ~0.1% (w/v). These solutions were then sealed in a high-pressure vessel (HP-500, CEM Corporation, Matthews, NC) and heated in a microwave (Mars 5, CEM Corporation, Matthews, NC). The vessels are designed to operate at temperatures and pressures up to 260°C and 33 bar respectively. The microwave automatically regulates energy input to reach the desired temperature within a pre-set time period, 2 min in this experiment, and holds this temperature for the required time. Microwave heating was carried out at 90, 100, 110 and 121°C for different durations from 90 s to 1 h. It took 4.5, 3 and 1.5 min, respectively, for the temperature to drop from 121, 110 and 100 to 90°C, the temperature shown not to cause thermal degradation of β-glucan for up to 3 h. The precise and continuous delivery of microwave energy ensures good reproducibility regardless of the amount of sample treated each time.

Molecular weight and molecular weight distribution were

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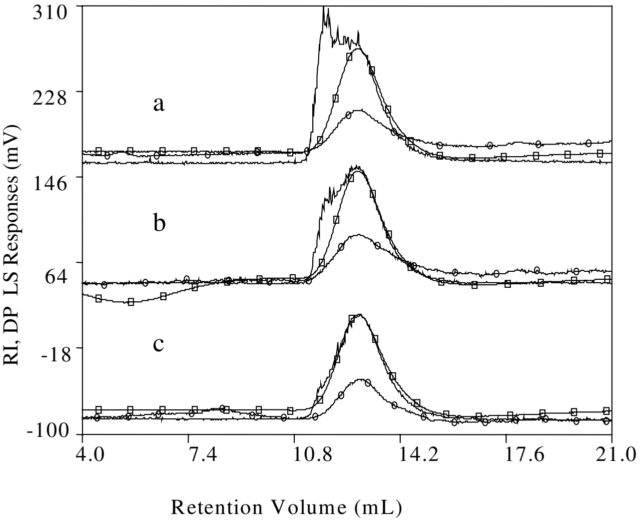

Fig. 1. HPSEC profiles of β-glucan solutions (0.1% w/v) after dispersal in water and heating at 80°C for 30 min and at 60°C for 3 h (a), and after heating by microwave at 100°C for 3 min (b) and 4 min (c); LS, DP and RI are the responses from light scattering (solid line), viscometry (□) and refractive index (○), respectively.

determined by high performance size exclusion chromatography (HPSEC) equipped with three detectors, a 90° laser LS detector, a differential viscometer (DP) and a refractive index (RI) detector (Viscotek, Houston, TX). Pullulan standards (P-82, JM Science Inc., NY) were used for calibration of the detectors. A value of 0.146 ml/g was used as RI increment. The chromatographic system was a Shimadzu SCL-10Avp unit (Shimadzu Scientific Instruments Inc., Columbia, MD) and used two columns in series: a Shodex Ohpak KB-806M (Showa Denko K.K., Tokyo), and a Ultrahydrogel linear (Waters, Milford, CT). The mobile phase was 0.1 M $NaNO_3$ with 0.03% (w/w) NaN_3 and the flow rate was 0.6 ml/min. The columns were maintained at 40°C.

3. Results and discussion

3.1. *Effect of microwave heating on dispersion of aggregates*

Fig. 1a shows the HPSEC profiles of β-glucan solution prepared by the standard procedure (i.e. 80°C for 30 min and 60°C for 3 h) from the three detectors. Although the solution was filtered (0.45 μm) before analysis, the shape of the LS peak indicated that the solution contained some large aggregates. There was a strong signal at the lower elution volumes where there is little response from either the DP or RI signals. This is because LS is more sensitive to particles of higher mass. The aggregates only take up a small proportion by weight, thus, low RI and DP signals are obtained. The small number of aggregated particles significantly raises the weight-average molecular weight (M_w) but has little effect on the number-average molecular weight (M_n), leading to a large polydispersity index (M_w/M_n). The average polydispersity index of β-glucan solutions without microwave heating was as high as 14.8 (Table 1). For a well-dissolved and non-degraded oat β-glucan, the polydispersity index falls in the range of 1.5 ± 0.5. For a given sample, a large polydispersity index is often evidence of molecular aggregation, which leads to poor reproducibility of molecular weight data and a high standard deviation.

Temperature was a critical factor in the effect of microwave heating of solutions of β-glucan. At 90°C or lower, the treatment did not disrupt the aggregates effectively even after 30 min. However, a suitable duration of microwave treatment at 100°C or higher produced satisfactory dispersion of aggregates. At 100°C, when treatment was less than 3 min, aggregation was reduced but not eliminated (Fig. 1b). This was reflected in the large standard deviation in the mean value of molecular weights of replicates, and in the large polydispersity index (Table 1). Fig. 1c represents the HPSEC profiles after a 4 min treatment, in which the LS signal showed a distribution corresponding to the RI and DP signals. The values for molecular weight, intrinsic viscosity, and polydispersity index for 4 and 10 min treatments were similar (Table 1) and the standard deviations were relatively small. The improved reproducibility suggested the elimination of aggregates. Both 3 and 10 min treatments at 110 and 121°C gave similar data and good reproducibility, indicating that 3 min was sufficient to disrupt most of the aggregates at temperature above 110°C.

The above results were obtained at a concentration of ~0.1% (w/v), as commonly used for HPSEC analysis. We have found (data not shown) that concentration has a large effect on the aggregation behaviour of β-glucan solutions.

Table 1
Molecular weight (M_w), intrinsic viscosity [η], polydispersity index (P_d) radius of gyration (R_g) and sample recovery after filtration (0.45μm) of β-glucan solutions heated by microwave at 100°C for different periods of time. Values are means of triplicates

Heating time (min)	$M_w \times 10^{-6}$ (Da)	[η] (dl/g)	R_g (nm)	P_d	Recovery (%)
0	7.3 ± 7.0	8.5 ± 0.3	78 ± 3	14.8 ± 18.0	75 ± 5
2	2.7 ± 1.8	8.0 ± 0.03	66 ± 4	6.0 ± 6.0	92 ± 3
2.5	2.3 ± 0.7	8.5 ± 0.4	74 ± 6	3.4 ± 2.5	92 ± 3
3	1.9 ± 0.3	8.3 ± 0.5	73 ± 3	2.2 ± 0.4	95 ± 2
4	1.7 ± 0.3	8.4 ± 0.3	73 ± 2	2.0 ± 0.4	98 ± 2
10	1.7 ± 0.1	8.7 ± 0.2	75 ± 2	1.7 ± 0.1	90 ± 3

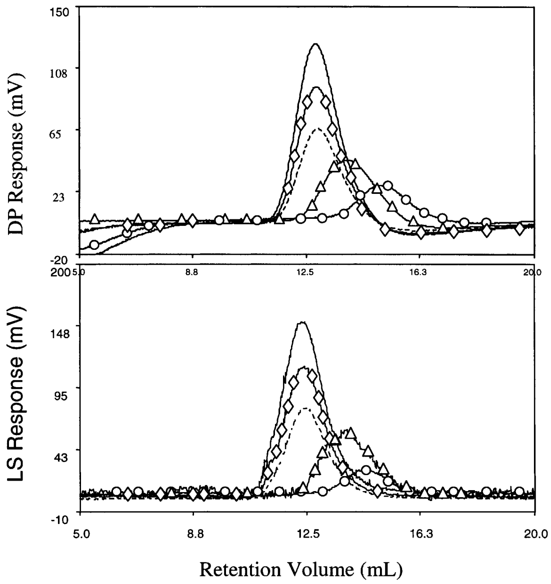

Fig. 2. HPSEC profiles (LS and DP) of β-glucan solution (0.1% w/v) after heating at 80°C for 30 min and 60°C for 3 h, centrifugation (10,000 g, 30 min) and filtration (0.45 and 0.22 μm, 3 ×) (solid line); and after heating by microwave at 121°C for 2.5 min (dashed line), 10 min (◇), 30 min (△) and 1 h (○).

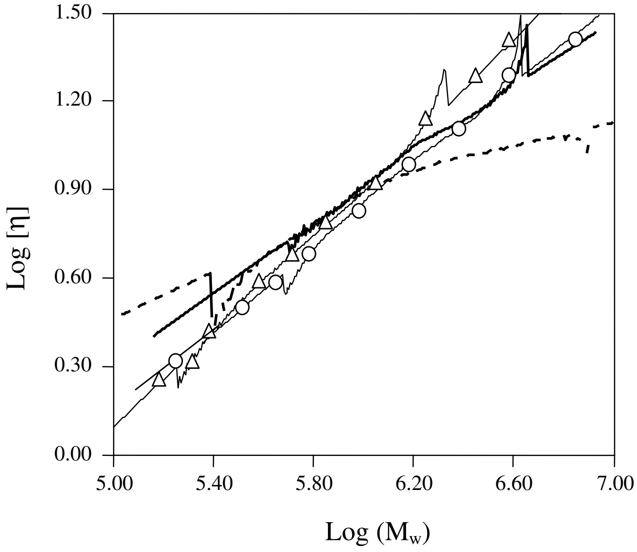

Fig. 3. Mark–Houwink plots for β-glucan solutions (0.1% w/v) after dispersal at 80°C for 30 min and 60°C for 3 h (dashed line); after centrifugation and filtration (solid line); after heating by microwave at 100°C for 10 min (○) and at 121°C for 30 min (△).

The higher the concentration, the greater the tendency to aggregate, and the longer the time required to disrupt the aggregates. There was little difference between different batches of samples; presumably the initial dissolution step minimised potential differences.

3.2. Assessment of polymer degradation and structural changes

To investigate possible degradation of β-glucan under selected conditions, sample solutions (0.1% w/v) were centrifuged at 10,000 g for 30 min and the supernatants were filtered three times (0.45 and 0.2 μm filters connected in series). These steps removed or disrupted (by shear during filtration) most of the aggregates; aggregates could no longer be detected by on-line LS. There is evidence from studies of thermal degradation of polysaccharides that the rate of depolymerisation does not depend on chain length (Zentz, Verchere & Muller, 1992). Thus, although some fractions of the original sample were removed, it is reasonable to use the remaining fractions to study stability. This minimises the difficulty of having to distinguish polymer degradation from dissociation of aggregates.

The viscosity and LS distributions for solutions treated at 121°C for 2.5 and 10 min did not show much difference from the original sample (Fig. 2). At all temperatures, so long as the duration of heating was not longer than 10 min there was no significant degradation. A slight difference between samples not heated by microwave and samples heated for less than 10 min, probably arose from further disruption of remaining low amounts of relatively small molecular aggregates, rather than from molecular depolymerisation. This is because the values for molecular weight and intrinsic viscosity of the same solution treated at different temperatures for 4–10 min converged to constant values ($1.6 \pm 0.2 \times 10^6$ g/mol and 8.5 ± 0.1 dl/g, respectively). As expected, temperature and duration of treatment had a combined effect on the stability of β-glucan. M_w was not affected by treatment at 100°C for up to 1 h. After treatment for 30 min at 121°C M_w declined from $1.6 \pm 0.2 \times 10^6$ g/mol to $1.3 \pm 0.2 \times 10^6$, and to $1.0 \pm 0.2 \times 10^6$ after 1 h at 110°C.

As shown in Fig. 3, the Mark–Houwink (MH) plots ($[\eta] = kM_w^\alpha$) of samples without microwave heating gave a very low value of exponent α (0.32 ± 0.08), corresponding to a compact structure. After removing large aggregates by centrifugation and filtration of the sample solutions, the exponent α increased to 0.60 ± 0.04; the exponent α for samples in which aggregation was effectively eliminated by microwave heating converged to 0.76 ± 0.04, a typical value for random coil polymers in a good solvent. This value is in good agreement with values reported by Böhm and Kulicke (1999) and Vårum and Smidsrød (1988). It indicates that the change in the value of exponent α was due to the elimination of molecular aggregation rather than a change in molecular structure.

3.3. Sample recovery

Sample solutions were usually filtered (0.45 μm) before HPSEC. The filtration process was difficult for samples prior to microwave treatment, indicating the existence of large particles in the solution. These large particles remained in the filter, rapidly reducing the effective pore size of the membrane, and leading to a low recovery of β-glucan. In general, microwave heating increased sample recovery, depending on the heating time and temperature. For example, β-glucan recovery after filtration was 75 ± 5% for a sample prepared by the 'usual' dissolution procedure and 90 ± 2% for the same sample heated by microwave at 90°C for 3 min, as measured by acid hydrolysis and high performance anion exchange chromatography (Cui, Wood, Weisz & Beer, 1999). The on-line RI detector also enables determination of sample recovery, supposing all injected mass was eluted at the assigned peaks. As shown in Table 1, after heating the solutions at higher temperatures and for a longer time, 95–98% recovery following filtration was achieved. The sample treated for 10 min at 100°C had lower recovery, probably as a result of film formation. A number of small fragments of transparent film fragments were seen floating in the solution after heating. The sample vessels were weighed before and after heating to confirm that there was no loss of water by evaporation. The basis for this phenomenon needs further investigation. There was no indication of molecular aggregation in the bulk solution after removal of the film fragments by filtration. It seems that the film formation was a result of local reactions, possibly on the surface of the solution.

4. Conclusions

Microwave heating in a high-pressure vessel improves the dispersion of β-glucan solutions in water without polymer degradation. For the β-glucan used in this study and similar preparations, treatments at 100–121°C for 4–10 min were adequate. The approach described in this paper may have applications in the molecular characterisation of similar water soluble polysaccharides.

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