Carbohydrate Research, 77 (1979) 191–204
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SOLUTION PROPERTIES OF WHEAT-FLOUR ARABINOXYLANS AND ENZYMICALLY MODIFIED ARABINOXYLANS

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(Received March 6th, 1978; accepted for publication in revised form, April 19th, 1979)

ABSTRACT

A series of water-soluble arabinoxylans has been prepared from purified wheat-flour arabinoxylan by partial removal of arabinosyl side-branches using an α-L-arabinofuranosidase. Their solubilities, molecular weights, intrinsic viscosities, and degrees of hydration were measured. The results suggest that the arabinoxylan assumes an extended rod-like conformation in solution. The solubilizing effect of arabinosyl substituents was not the result of increased hydration, but due to their ability to prevent intermolecular aggregation of unsubstituted xylose residues. The extended conformation of arabinoxylan is consistent with its known organization and function in plant cell-walls.

INTRODUCTION

Wheat-flour arabinoxylans are polysaccharides having (1→4)-linked β-D-xylopyranose backbones substituted[1] at intervals by single arabinofuranosyl groups at O-2 or O-3. The removal of L-arabinofuranosyl groups from the arabinoxylan by mild, acid hydrolysis[1] or enzymically by using α-L-arabinofuranosidase[2,3] produces an insoluble xylan. As the insolubility of (1→4)-β-D-xylans is due to their ability to aggregate[4], it has been presumed that the arabinosyl substituents are responsible for the solubilizing effect[5]. To investigate how the arabinosyl substituents solubilize the xylan backbone, the changes in solution properties of wheat-flour arabinoxylans (following partial removal of arabinosyl substituents by using a purified α-L-arabinofuranosidase) have been analysed. Solutions of wheat-flour arabinoxylans have high intrinsic viscosities compared with those of other neutral polysaccharides, and the cause of this anomaly has also been studied.

MATERIALS AND METHODS

Monosaccharide analysis. — Polysaccharide samples were hydrolysed at 100°

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for 4 h in 0.5M HNO_3 containing 0.5% (w/v) urea in a sealed tube[6]. The monosaccharide composition of the hydrolysates was determined by g.l.c.[7].

Preparation and properties of wheat-flour arabinoxylans. — Wheat, *Triticum aestivum* L. cv. Insignia, was pearled and milled[7], and the arabinoxylan was isolated by a procedure which essentially followed that used in earlier work[8]. The yield was 0.18% of the pearled grain and 0.22% of the flour. This product, referred to as stock arabinoxylan, was stored in a freeze-dried form over $CaCl_2$ at room temperature.

The protein content was estimated[9] with bovine serum albumin (Commonwealth Serum Laboratories, Melbourne, Australia) as the standard, and also from its nitrogen content (Australian Microanalytical Service, University of Melbourne). A protein value of 2.3% was obtained and the nitrogen content was 0.47%, corresponding[10] to 2.7% of protein (%N × 5.7).

The stock arabinoxylan yielded arabinose and xylose on hydrolysis, but no glucose or galactose. The ratio of xylose to arabinose (X/A ratio) for the stock arabinoxylan was 2.00 ± 0.05 (average of 15 separate determinations).

Substrates. — Xylan for use in enzyme assays was prepared from wheat straw[11]. The polysaccharide was extracted with 12% aqueous NaOH[12], and $NaBH_4$ (1 mg/ml) was added to prevent alkaline degradation[13,14]. The xylan was purified by repeated precipitations with Fehling's solution[15,16]. To lower the arabinose content (5–7%) of the wheat-straw xylan, samples of xylan (1%) were suspended in 0.125M oxalic acid and heated in a boiling water-bath for 1 h. After neutralisation with 3% NaOH, the xylan was reduced[17] with $NaBH_4$, to lower the substrate blanks in reductometric enzyme-assays. These xylan samples were designated O.A.X., and contained ~1% of arabinose.

Sodium carboxymethylxylan (CM-xylan) for viscometric assays was prepared[18] at a reaction temperature[19] of 55°.

o-Nitrophenyl β-D-xylopyranoside was purchased from P-L Biochemicals Inc., Wisconsin, U.S.A.

Enzyme assays. — The buffer used for all assays was 100mM sodium acetate adjusted to pH 4.0 with 100mM acetic acid, and contained 250mM NaCl and 5mM EDTA[20]. All assay mixtures were incubated at 40°.

Wheat-flour arabinoxylan served as a substrate for reductometric arabinofuranosidase assays. When crude enzyme preparations were assayed with this substrate, xylose and oligosaccharides were also liberated. However, paper chromatography showed that the purified enzyme fraction removed only arabinose residues. The incubation mixture contained a 0.4% solution of arabinoxylan and buffer to a total volume of 1 ml. After a 2-h incubation, the reaction was terminated by the addition of 1 ml of copper reagent[21], and the reducing sugars were determined[22]. A boiling time of 10 min was used and the results were expressed in xylose equivalents.

For the reductometric xylanase assay, the conditions were the same, except that 0.75 ml of a 10 mg/ml suspension of O.A.X. was used as the substrate, and samples were centrifuged prior to absorbance measurements.

A viscometric assay was used to detect endo-(1→4)-β-D-xylanase activity.

Flow-times of the enzyme–substrate mixtures were measured in a No. 1 B.S.S. Ostwald viscometer which had been modified for use with a volume of 2.0 ml. CM-xylan solution (2.5% w/v, 2.2 ml) and 0.55 ml of enzyme were equilibrated separately at 40°, and then mixed in a test tube at $t = 0$. A 2.0-ml sample of this incubation mixture was placed in the viscometer and the flow-time observed. The sample was then transferred back into the test tube and left at 40°. The flow-time of a 2.0-ml sample was recorded again at $t = 21.5$ h.

For the assay employing *o*-nitrophenyl β-D-xylopyranoside as the substrate[23], the reaction mixture contained 0.4 ml of substrate solution (mM), and enzyme solution and buffer to a volume of 3 ml. The reaction was terminated by the addition of 1 ml of M Na_2CO_3 solution after incubation for 2 h. Activity was expressed as the increase in absorbance at 420 nm.

α-L-Arabinofuranoside arabinofuranohydrolase (*α-L-AF*). — The commercial enzyme-preparation Pectinol 59-L (Rohm and Haas Co., Philadelphia, Pa. U.S.A.) was used as the source of α-L-AF (EC 3.2.1.79).

Pectinol 59-L samples (10–15 g of the supplied solution) were diluted to ~60 ml with distilled water, kept at 6°, and then centrifuged to remove insoluble material. The supernatant solution was passed through a UM-10 ultrafiltration membrane (Amicon Corporation, Massachusetts, U.S.A.), to remove contaminants of low molecular weight, and then freeze-dried. The α-L-AF was fractionated on a column (33 cm × 2 cm^2) of DEAE-Sephadex A-50 (Pharmacia) which had been pre-cycled and equilibrated with a 10mM sodium hydrogen maleate–NaOH buffer (pH 6.0) containing 50mM NaCl and 0.02% of NaN_3. A 65-mg sample of the freeze-dried, filtered enzyme was applied to the column. Approximately one bed-volume of buffer was washed through the column, and then a linear gradient of NaCl (220 ml) in the 10mM maleate buffer was applied to a final NaCl concentration of 200mM. Washing with 200mM NaCl was continued for a further 95 ml. Chromatography was performed at 6°, and the NaCl gradient was monitored by conductivity measurements.

The fractions with most activity on arabinoxylan, but with little activity on CM-xylan, xylan, or *o*-nitrophenyl β-D-xylopyranoside, were combined, and their action pattern on arabinoxylan and O.A.X. was examined by descending paper chromatography with 1-butanol–pyridine–water[24] (6:4:2.5). Sugars were detected with alkaline $AgNO_3$[25] and fixed with 10% aqueous sodium thiosulphate. When the combined enzyme fractions were incubated with either arabinoxylan or O.A.X., only arabinose was produced. Longer incubations (24 h) with either polysaccharide produced, in addition to arabinose, small proportions of xylose and an oligosaccharide (probably an arabinosyldixylose)[24]. After a 100-min incubation of the combined enzyme fractions with stock arabinoxylan, the small decrease in molecular weight (65,000 to 60,000) was fully accounted for by the loss of arabinose.

Preparation of arabinoxylans having diminished arabinose content. — The incubation mixture consisted of 378 ml of stock arabinoxylan solution (3.7 mg/ml) in acetate buffer (pH 4.0), to which was added a further 300 ml of the same buffer. The substrate solution in buffer was brought to 40°, enzyme solution (22 ml) then

added, the solution thoroughly mixed, and a 100-ml portion withdrawn immediately and kept in a boiling water-bath for 15 min to ensure enzyme inactivation. This sample is referred to as aX-0. Further 100-ml samples (aX-1–6) were withdrawn at intervals of 50 min, and each was inactivated as described for aX-0. Only the aX-6 solution showed cloudiness after the boiling treatment. Each sample was dialysed against distilled water in the presence of toluene, recovered by freeze-drying, and stored over $CaCl_2$. The samples showed a steady decrease in arabinose content and covered a suitable range of X/A ratios for use in subsequent studies. The presence of inactivated enzyme increased the protein content of each sample by ~0.5%, so that samples finally contained ~3% of protein.

Solubility analyses. — Samples of the arabinoxylan series were dispersed thoroughly in distilled water (~3 ml) by stirring for 5 days in the presence of small amounts of chloroform or toluene. The initial concentration of polymer in each sample (~8.5 mg/ml) was determined accurately either by using a differential refractometer (Brice–Phoenix, The Virtis Co., Gardiner, N.Y., U.S.A.) for soluble samples, or by hydrolysing an aliquot of polymer suspension and determining the concentration of reducing sugars produced. A correction was applied for losses in sugars during the hydrolysis procedure, based on the recovery of free sugars treated identically.

The samples were centrifuged at 12,000*g* for 10 min and the supernatant solutions collected ("soluble fraction"). The sedimented fractions were washed twice with one volume of distilled water, and the final "insoluble fraction" was collected by freeze-drying. Solubility was expressed as the ratio of the concentration of polymer in the "soluble fraction" to the initial concentration of the suspension. X/A ratios of the "soluble" and "insoluble" fractions were determined by g.l.c.

Degree of hydration. — A low-temperature n.m.r. method[26] was used to determine the degree of hydration of the arabinoxylan samples. This method measures the amount of water "unfrozen" at −30° and yields the same values for the degree of hydration of proteins as obtained by conventional procedures[27]. It has also been demonstrated that the procedure is equally valid when applied to polysaccharides[28]. Arabinoxylan solutions (~1%) and crystallised bovine serum albumin (~3%) (Sigma Chemical Co., Missouri, U.S.A.) were made up in distilled water. The concentrations of the arabinoxylan solutions were determined by differential refractometry. The concentrations of bovine serum albumin was determined spectrophotometrically by using an extinction coefficient at 280 nm ($E^{1\%}_{1cm}$) of 6.65.

N.m.r. measurements were made at −30° in a JEOL PFT-100 Fourier-transform spectrometer equipped with a JNM-VT-3C temperature controller. The advantages of using a Fourier-transform spectrometer for this work, and the precautions necessary, have been discussed elsewhere[28].

The hydration[29] of BSA of 0.40 g/g was used as the standard to calculate absolute hydrations. The operating conditions employed were: pulse width 19 μsec (flip angle of almost 90°), 200 pulses, 0.12-sec repeat time, and a 10-kHz spectral width.

Viscometry. — Viscosity was measured at 25.0 ± 0.02° in a Cannon–Ubbelohde Four Bulb Shear Dilution Viscometer (Cannon Instrument Co., Pennsylvania, U.S.A.).

A stock solution (0.5 g/dl) of each arabinoxylan sample was made up in 0.01M NaCl and dialysed against this solvent. The solution and solvent were then filtered through a membrane filter (Millipore Corporation, Massachusetts, U.S.A.) of pore size 3 μm. The concentration of the arabinoxylan solution was determined by differential refractometry. The intrinsic viscosity was determined by using the Huggins and Kraemer equations[30].

After viscometry, each solution was dialysed against distilled water, the arabinoxylan recovered by freeze-drying, and the X/A ratio determined by g.l.c.

Molecular size determinations. — Gel-filtration chromatography on Sepharose 2B and 4B columns was used to measure the molecular size of the stock arabinoxylan. The void volume (V_0) was determined by eluting 20 mg of *E. coli* cells (Miles–Seravac) in 1 ml of 10mM Na_2HPO_4 buffer (pH 6.8) with 0.3% aqueous NaCl containing 0.05% of NaN_3, monitoring the absorbance at 500 nm. The total bed-volume was determined from the elution volume of phosphate[31]. For the Sepharose 2B column, a solution of dextran ($M_w > 10 \times 10^6$) was used to determine V_0.

Samples of the stock arabinoxylan (8–12 mg) for chromatography were shaken in 1 ml of 10mM Na_2HPO_4 buffer (pH 6.8) at room temperature for ~3 h, to allow complete dissolution. The solubilisation process could be accelerated by heating, but such treatment was avoided throughout this work because changes of solution properties have been reported after heating solutions of arabinoxylans[32,33]. Fractions were assayed for carbohydrate by the phenol–sulphuric acid method[34] and for protein[9] by using BSA as the standard.

Molecular weight determinations. — Solutions of stock arabinoxylan (~10 mg/ml) for partial specific volume and sedimentation measurements were made up in distilled water and in 0.01M NaCl, and each was dialysed against its respective solvent. The concentrations of the solutions were determined by differential refractometry and the density was determined by using a Digital Precision Density Meter DMA 02C (Anton Paar, K.G., Austria). The partial specific volume of the stock arabinoxylan in distilled water was 0.588 ±0.003 ml/g and, in 0.01M NaCl, 0.596 ±0.003 ml/g.

Conventional, sedimentation equilibrium ultracentrifugation was performed at 9,000 r.p.m. on a Beckman Model E Analytical Ultracentrifuge. Interference optics were used to obtain the sedimentation equilibrium data, and photographs were recorded on Kodak spectroscopic plates. The photographic plates were measured on a Nikon Microcomparator (Nippon Kogaku K.K. Tokyo, Japan).

The temperature for the sedimentation equilibrium runs was 20° and the solution concentration was 2 mg/ml. For convenience, runs were left at speed for 30 h, allowing sufficient time for equilibrium to be reached. This was confirmed by the constancy of the fringe pattern after an additional 5 h.

The weight-average molecular weight was calculated from the number of interference fringes crossed at equilibrium (Δj_{eq}), using Δj_{sb} (number of fringes crossed in the synthetic boundary cell) calculated from the refractive index increment[35].

RESULTS AND DISCUSSION

The *Aspergillus niger* arabinofuranosidase obtained by fractionation of the crude enzyme preparation did not completely remove the arabinofuranosyl residues from the wheat arabinoxylan. When only 18% of the total arabinose had been removed, arabinose release stopped. The arabinofuranosidase was not inactivated under the conditions of the incubation, and product inhibition was not the reason for the cessation of hydrolysis. It has been reported that a fractionated Pectinol R-10 preparation acting on wheat arabinoxylan for prolonged periods produced a xylan with only traces of arabinose[2,3]. It is significant that one of these arabinofuranosidase preparations[2] was able to remove 90% of the L-arabinose residues from a wheat-flour arabinogalactan[36], whereas the preparation used in this work removed no significant amounts of arabinose from a similar wheat-arabinogalactan–peptide[37]. This finding suggests a specificity difference between the two preparations.

Following the graded, enzymic hydrolysis, the arabinoxylan fraction aX-6 was the only fraction not completely soluble after boiling the solution and cooling. However, when the polymers were recovered by freeze-drying, only aX-0, aX-1, and aX-2 were completely soluble. Fig. 1 shows the progressive change in solubility of the arabinoxylan samples as a function of X/A ratio. As the X/A ratio increased, there was an abrupt change in solubility between ratios of 2.30–2.35, and thereafter the solubility did not change, although the X/A ratio increased to 2.55.

All of the arabinoxylan remained soluble after incubation with the enzyme for 100 min and showed a slight increase in X/A ratio. After 150 min, an "insoluble fraction" separated, and this fraction showed the expected, progressive increase in X/A ratio (Fig. 2). The "soluble fraction", on the other hand, showed a slight

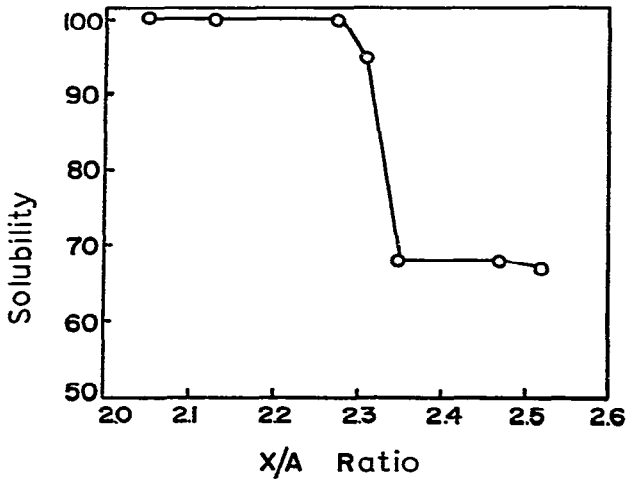

Fig. 1. Relationship between the solubility and X/A ratio of the arabinoxylan samples (aX-0 to aX-6) derived from stock arabinoxylan by hydrolysis with α-L-AF for increasing time-intervals. See Materials and Methods for details of the solubility determination.

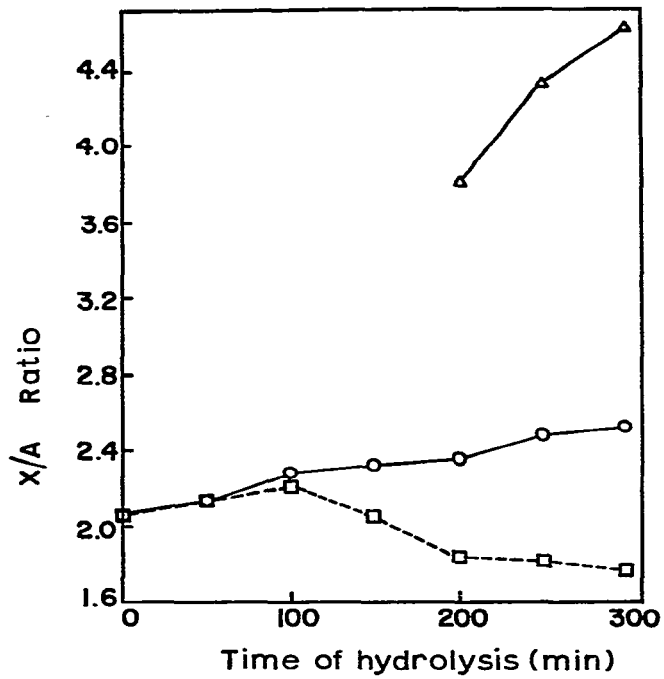

Fig. 2. Relationship between X/A ratio and time (min) of hydrolysis of the arabinoxylan with α-L-AF. The total sample (aX-0 to aX-6) derived from the stock arabinoxylan is shown (○), as well as the "soluble fraction" (□) and the "insoluble fraction" (△).

decrease in X/A ratio from 100 to 200 min and thereafter the ratio remained constant.

This pattern of change suggests that the original arabinoxylan is heterogeneous with respect to the molecular species present. One fraction, representing ~65% of the total, remained soluble throughout the incubation and is apparently not susceptible to the arabinofuranosidase action. The remaining arabinoxylan is susceptible to enzymic hydrolysis and, with the progressive loss of arabinose residues, becomes insoluble.

There are several sources of molecular heterogeneity which might affect susceptibility to arabinofuranosidase action.

(*a*) The position of arabinofuranosyl substitution of the xylan backbone. It has been shown[1] that 33% of the arabinose substituents are linked through O-2, the remainder being linked through O-3. If the arabinofuranosidase specifically hydrolysed only one type of arabinofuranosyl linkage and this predominated on some chains, then their cleavage would result in specific insolubilization of these chains.

(*b*) The arrangement of the arabinofuranosyl linkages on the xylan backbone. Arabinofuranose residues present in sequences of contiguous, substituted xylosyl residues may be resistant to hydrolysis, or, alternatively, disubstituted xylosyl residues may be resistant to hydrolysis, so that molecules with high proportions of

TABLE I

HYDRODYNAMIC CHARACTERISTICS OF THE ARABINOXYLAN SAMPLES

Sample	*X/A ratio*	*Intrinsic viscosity (dl/g)*	*Hydration (g/g)*	*Simha shape-factor*[a] *(ν)*	*Axial ratio*[49,50] *(a/b)*
Stock arabinoxylan	2.00	6.12	0.23 ± 0.04	740 ± 35	140 ± 10
Stock arabinoxylan–boiled	2.00	5.58	0.33 ± 0.03	600 ± 20	125 ± 5
aX-0	2.03	5.38	0.42 ± 0.05	530 ± 25	115 ± 5
aX-1	2.11	5.14	0.51 ± 0.14	460 ± 65	105 ± 10
aX-2	2.21	4.26	0.31 ± 0.07	470 ± 35	105 ± 5
aX-3 "soluble fraction"	2.10	4.09	0.46 ± 0.04	390 ± 15	96 ± 5
aX-4 "soluble fraction"	1.83	3.70	0.35 ± 0.04	390 ± 15	98 ± 5
aX-5 "soluble fraction"	1.86	4.09	0.30 ± 0.04	460 ± 20	105 ± 5
aX-6 "soluble fraction"	1.75	4.11	0.35 ± 0.05	430 ± 25	100 ± 5

[a]Calculated from Equation *1*, using a partial specific volume of 0.596 ml/g (See Materials and Methods).

one or both of these features would remain unchanged, whereas molecules having fewer of these features would be susceptible and be rendered insoluble.

Characterization of the "soluble" and "insoluble" fractions by methylation and periodate oxidation will be necessary to decide between these possibilities.

Although the "insoluble" fractions progressively lose arabinosyl substituents, even here the hydrolysis does not proceed to completion. This situation may be due, in part, to the increasing insolubility of the arabinoxylan molecules, resulting in lowered accessibility to the enzyme, and in part to the presence of resistant features of the types mentioned above.

Degree of hydration. — The hydration values for the "soluble" portions of each arabinoxylan sample are given in Table I. As a control, a sample of stock arabinoxylan was boiled for 15 min under the same conditions used to inactivate the arabinofuranosidase in the enzyme-treated samples (stock arabinoxylan–boiled). The degrees of hydration of all the arabinoxylan samples having a similar thermal history were not significantly different when the experimental error is considered. The average value was 0.38 g/g.

The unboiled, stock arabinoxylan, however, had a significantly lower hydration than all of the other samples. The observed increase in hydration of the boiled arabinoxylans is comparable with that reported[38] for a sulphated algal polysaccharide on heating its solution to 90°.

The experimentally determined hydration values for the arabinoxylans are similar to those of most proteins and indicate that the main cause of the high viscosity of arabinoxylan solutions is not a high degree of hydration. Hydration values were not apparently correlated with the extent of arabinose substitution. However, the errors in the hydration values for all the arabinoxylans were large, and small differences in hydration would not have been detectable. The present evidence suggests that L-arabinofuranose residues do not contribute to the specific water-binding

properties of arabinoxylans, although they could be important for non-specifically bound water (*i.e.*, osmotic in origin). This type of bound water may be unaffected by the removal of only a fraction of the L-arabinofuranose residues present. The "hydrophilic character" suggested for arabinofuranosyl homopolymers[39] may well be due to such non-specifically bound water.

The degrees of hydration of some other polysaccharides have also been determined in this laboratory[28], and these values were similar to that of unboiled, stock arabinoxylan (0.23 g/g). Seed galactomannans[40], wheat arabinogalactan–peptide[8], and dextran (Sigma Chemical Co.; molecular weight, 83,000) had hydrations of 0.11, 0.10, and 0.20 g/g, respectively. Other values for the degree of hydration of polysaccharides in the literature are sparse, but a figure of 0.3 g/g has been reported for Sephadex[26], and values of 0.35 and 0.56 have been estimated[41] for agarose gels.

Viscosities. — The intrinsic viscosities for the nine arabinoxylan samples analysed and the X/A ratio of each sample are also listed in Table I. None of the polymer samples showed shear-dependence. All samples yielded a Huggins constant, k', in the range of 0.26–0.61, with an average value of 0.46.

The intrinsic viscosity of stock arabinoxylan in 0.01M NaCl (6.12 dl/g) was similar to that recorded in distilled water (6.08 dl/g) and is somewhat higher than the figures reported previously for other wheat-flour arabinoxylan preparations (1.76 dl/g[8], 2.5 dl/g[42], and 0.8–3.1 dl/g[43]). The lower values reported by these workers could relate to differences in molecular size and thermal history, and, especially, to the concentration range used in obtaining the viscosity data[44].

Compared with the intrinsic viscosity of solutions of other neutral polysaccharides, such as dextran[45] (0.214 dl/g) and beet arabinan[3] (0.195 dl/g), arabinoxylan solutions are much more viscous. The value recorded for stock arabinoxylan in this study is relatively high for an uncharged polysaccharide, but not as high as those reported[40] for seed galactomannans (10–14 dl/g) which have similar basic structures and solution properties.

Stock arabinoxylan had a significantly decreased, intrinsic viscosity, $[\eta]$, after it had been boiled (5.58, *cf.* 6.12 dl/g). From Equation *1*, it is apparent that a substantial decrease in the Simha shape-factor[46], ν, must accompany this treatment, to over-ride the increase in the degree of hydration (δ) which is also induced by boiling (0.23 to 0.33 g/g).

$$[\eta] = \nu(\bar{v}_2 + \delta v_1^o), \qquad (1)$$

where $\bar{v}_2$ is the partial specific volume of the polymer, and v_1^o is the solvent specific volume.

Calculation of the Simha shape-factor for stock arabinoxylan and stock arabinoxylan–boiled shows the latter to have a much smaller value for ν (Table I). Thus, these samples exhibit significantly different shapes in solution. The thermal history of an arabinoxylan sample is, therefore, an important determinant of its solution conformation.

A linear dependence of intrinsic viscosity upon X/A ratio was found within

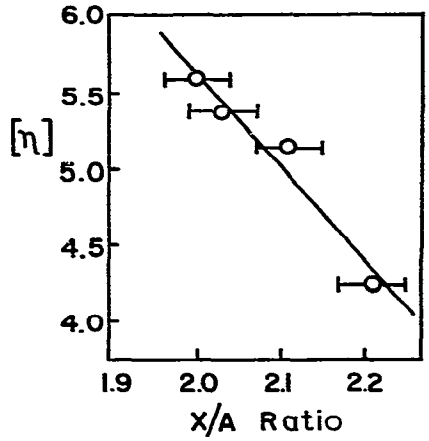

Fig. 3. Relationship between the intrinsic viscosity, [η](dl/g), and X/A ratio for stock arabinoxylan–boiled (X/A = 2.00) and α-L-AF-hydrolysed samples, aX-0 to aX-2.

the series of stock arabinoxylan–boiled, aX-0, aX-1, and aX-2 (Fig. 3). Viscosity decreased as the arabinose content was lowered. Seed galactomannans show a similar decrease in viscosity with the progressive removal of side-branches[40,47]. Since the arabinoxylan samples in the series with X/A ratios from 2.00–2.21 have the same molecular weight (aX-2 = 60,000 ±6,000; *cf.* stock arabinoxylan, 65,000 ±6,500) and intrinsic viscosity decreased within the series, a decrease in hydration or a change in conformation of the arabinoxylan was indicated (Equation *1*). The degree of hydration for these four arabinoxylan samples is relatively constant, while the axial ratio decreases continuously as the arabinose content decreases. It therefore appears that, for the soluble arabinoxylans, the removal of arabinosyl side-chains causes a decrease of asymmetry.

For the water-soluble portions of the samples aX-3, aX-4, aX-5, and aX-6,

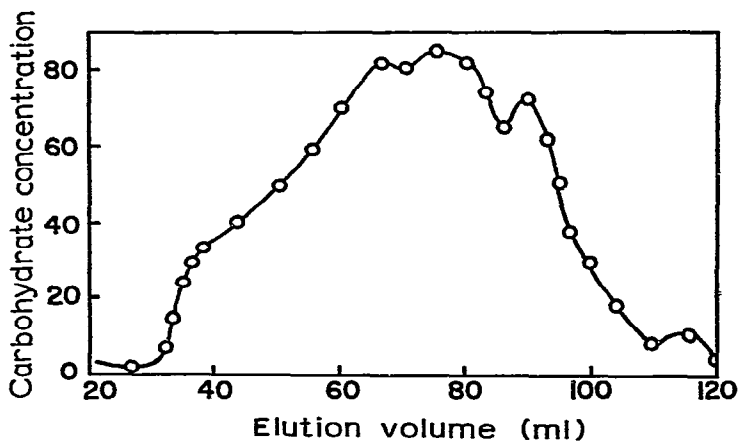

Fig. 4. Elution profile after gel filtration of stock arabinoxylan on a column (46 × 2.5 cm) of Sepharose 2B using a flow-rate of 7 ml/h. The void volume was 32 ml and the total bed-volume was 110 ml.

there was no correlation between viscosity and arabinose content (Table I). These fractions would be expected to have intrinsic viscosities greater than stock arabinoxylan–boiled, since their X/A ratios are less than 2.00. However, these "soluble fractions" may represent molecules from the lower end of the molecular-size distribution of the parent fraction; hence their molecular weights may be lower than 65,000, and their intrinsic viscosities would be correspondingly lower. Measurements of the molecular weights of all the "soluble" aX fractions would be needed to confirm this conclusion.

Molecular size and conformation. — Elution profiles for the chromatography of stock arabinoxylan on Sepharose 4B and Sepharose 2B (Fig. 4) indicate that the polysaccharide sample had, as previously reported[8,33], a broad distribution of molecular size. It appears that some of the stock arabinoxylan molecules are of very high molecular weight, or have an asymmetric conformation, since they have high, apparent molecular weights on gel-filtration media calibrated with dextrans. The weight-average molecular weight of stock arabinoxylan was determined in the ultracentrifuge as 65,000 $\pm$6,500. This value is very much lower than that indicated from gel filtration, but is consistent with most reported molecular weights of other arabinoxylan samples (22,000–119,000[42], 38,800[48], and 70,000[1]). Thus, it might be predicted that the molecules have an asymmetric conformation. This conclusion is supported by calculations (Table I) using values of the Simha shape-factor obtained from Equation *1*. From these, the axial ratio (a/b) can be determined[49,50].

A calculation of the maximum possible axial ratio can be made, knowing the weight-average molecular weight (65,000). As two-thirds of the molecule (*i.e.* ~44,000) will be composed of xylose residues, the average number of xylose residues per molecule is ~330. Using the value of 5Å for the length of a xylose residue[51], the length of a fully extended xylan-backbone is calculated to be 1650 Å. Arabinose side-branches may project from either side of this backbone [(1→2)- or (1→3)-linked to xylose], giving the molecule a minor axis (b) of between 10 and 15 Å. Assuming the polymer to be fully extended, the estimated axial ratio for an arabinoxylan of molecular weight 65,000, using a minor-axis value of 12.5 Å, is 132, which compares favourably with the value determined for the stock arabinoxylan molecule. From the shape factors calculated for each sample, it can be suggested that the polymers exist in a prolate, ellipsoidal conformation, rather than as oblate ellipsoids. The axial ratios for oblate ellipsoids, corresponding to ν values between 390–740, would be greater than the maximum a/b possible for this arabinoxylan[52] (*i.e.*, 132).

The experimentally determined axial-ratio for stock arabinoxylan of 140 suggests that this polymer has a very asymmetric solution-conformation, and, in fact, is fully extended. It contrasts with the earlier idea[53] that wheat hemicelluloses (X/A = 2.8) were best represented as linear polymers that form random coils in solution. At that time, it was known[53] that arabinoxylans had high frictional ratios, but whether this was due to asymmetry or a high degree of hydration could not be resolved without the necessary hydration data.

The heat treatment applied to all the arabinoxylan samples brought about a

change in conformation of the arabinoxylan to a slightly less-asymmetric form, as shown by the fact that stock arabinoxylan–boiled has a smaller axial ratio (125) than stock arabinoxylan (140). The reason for this conformational change is unknown.

The arabinose side-branches, which are attached, on average, to every second xylose residue in stock arabinoxylan, probably maintain the xylan backbone in a fully extended condition (Fig. 5*a*). However, after removal of some of the arabinosyl side-branches, the polymer appears to become more flexible (Fig. 5*b*), as indicated by the decrease of asymmetry accompanying a decrease of arabinose content of the soluble polymers. Upon removal of ~15% of the arabinosyl side-chains (Fig. 1), unsubstituted sections of the xylan backbones may approach closer and form stable, inter-chain associations and consequently produce insoluble aggregates (Fig. 5*c*). The association of unsubstituted xylan segments of arabinoxylans to form molecular aggregates is consistent with the known properties of xylans[39]. For sugar-cane hemicellulose, substituted xylan segments can also form intermolecular associations, but the unbranched sections tend to associate first[54].

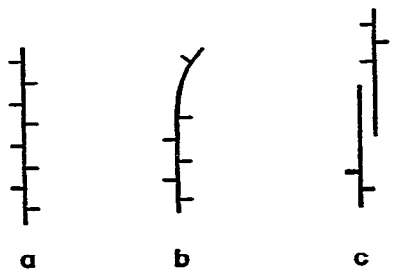

Fig. 5. Diagrammatic representation of the structure of arabinoxylan at varying X/A ratios. Arabinose side-chains are indicated as small branches to the xylan backbone. (*a*) Rod-like structure at X/A = 2.0; (*b*) decreased asymmetry of the soluble fractions after removal of some of the arabinosyl side-chains; (*c*) intermolecular, insoluble aggregates of arabinoxylan chains after extensive removal of arabinosyl side-chains.

Structure–function relationships. — The extended conformation of arabinoxylans in aqueous solution can be related to their arrangement and function in cell walls. The water-soluble arabinoxylan (stock arabinoxylan) studied in this work was isolated from wheat endosperm, where it occurs in cell walls together with cellulose and small proportions of glucomannan[7,33]. It has been shown by transmission electron-microscopy[7] that removal of the water-soluble arabinoxylans from cell walls partially exposes the microfibrillar structure. It appears that at least some of the water-soluble arabinoxylans are layered directly on to the surface of the cellulose microfibrils. It has been demonstrated that strong, non-covalent bonding occurs *in vitro* between barley aleurone arabinoxylan-chains and cellulose fibrils[55]. Such aggregations, as well as associations between arabinoxylans themselves[54], are considered to be responsible for the strength of cell walls[55]. The extended conformation of arabinoxylans demonstrated in this work is consistent with their ability to bind to cellulose microfibrils by an alignment process similar to that observed within

the cellulose microfibrils themselves, *i.e.*, a system of extended molecules held together by intermolecular hydrogen-bonding.

The non-starchy polysaccharides from wheat flour, which are predominantly arabinoxylans, increase the viscosity of dough[56] and also absorb large amounts of water relative to other dough constituents[57]. In this way, they have an important effect on the mixing characteristics of dough. The arabinoxylans make the major contribution to dough viscosity compared with all the other components of the pentosan fraction[58,59]. It has been shown in this work that the arabinoxylans form viscous solutions due to their asymmetric conformation. They do not have a high degree of hydration, as judged by n.m.r. measurements, nor are they of high molecular weight. It therefore appears that arabinoxylans exert their effect on the viscosity of dough by virtue of their extreme asymmetry, and not because of the extent to which water is associated with individual arabinoxylan molecules. However, the effect of arabinoxylan–protein associations, cross-linked arabinoxylan–protein complexes, and cross-linked arabinoxylan–arabinoxylan complexes, on the amount of water "bound" by doughs, remains unknown.

ACKNOWLEDGMENTS

We thank Mr. K. Mander (Cereal Chemistry Section, Victorian Department of Agriculture) for supplying and milling the wheat samples, and Dr. K. G. Granath (Pharmacia, Uppsala) for the dextran sample. Access to the ultracentrifuge and associated equipment at several C.S.I.R.O. divisions is gratefully acknowledged, as is the use of the spectropolarimeter and n.m.r. spectrometer in the Chemistry Department, La Trobe University. We also thank Dr. W. Sawyer for helpful discussions and suggestions regarding the n.m.r. hydration studies. One of us (K.A.A.) acknowledges the receipt of a Commonwealth Post-Graduate Research Award. This work was supported by the Australian Research Grants Committee and the Wheat Industry Research Council.

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