POLYSACCHARIDES OF TROPICAL GRASS SPECIES

III. SOLUTION PROPERTIES OF HEMICELLULOSE B FROM *Setaria sphacelata**

N. W. H. CHEETHAM†‡, (THE LATE) R. J. MCILROY†, AND D. J. WINZOR§

Departments of Agriculture† and Biochemistry§, University of Queensland, St. Lucia, Queensland 4067 (Australia)

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ABSTRACT

The solution properties of hemicellulose B from *Setaria sphacelata* have been investigated by equilibrium sedimentation and moving-boundary electrophoresis. Values of 40,000, 46,000, and 94,000 have been obtained for the apparent number-, weight-, and *z*-average molecular weights, respectively, of 0.4% polysaccharide solutions in 0.05I acetate, pH 5.3. Furthermore, the hemicellulose fraction is thought to consist almost entirely of polysaccharide molecules having molecular weights in the vicinity of 38,000, together with traces of much heavier material. Detailed boundary-analysis of the single, symmetrical, electrophoretic peak observed in the same medium indicated a fairly narrow mobility-distribution, at least 80% of the hemicellulose possessing a mobility of $-0.7 \pm 0.2 \times 10^{-5}$ $cm^2 sec^{-1} volt^{-1}$.

INTRODUCTION

The structures of hemicelluloses derived from grasses have received widespread attention, but their properties in solution have been studied less extensively. This communication presents some physicochemical properties of aqueous solutions of hemicellulose B from *Setaria sphacelata*, the chemical structure of which is very similar to those of the arabinoxylan polysaccharides of the various temperate[1] and tropical[2,3] grass species that have been examined.

RESULTS AND DISCUSSION

Chemical studies on *Setaria* hemicellulose B, done in conjunction with those on the corresponding polysaccharide from *Cynodon plectostachyus*[3,4], indicated a backbone of (1→4)-β-D-xylan with arabinose attachment or branching at the C-3 position and occasional linkage of 4-*O*-methyl-D-glucuronate to C-2 of the xylan chain[3]. In these respects there is marked similarity between the chemical nature of

*Taken, in part, from a thesis submitted by N. W. H. Cheetham to the University of Queensland in partial fulfilment of the requirements for the degree of Ph.D.

‡Present address: Department of Chemistry, James Cook University of North Queensland, Townsville, Queensland 4810.

the arabinoxylan fraction from these two sources, and, indeed, from all grass species examined[1–4]. Since the *Setaria* hemicellulose was known to be electrophoretically "pure" to the extent that a single peak was observed in moving-boundary electrophoresis[5], this polysaccharide was subjected to further physicochemical characterization in order to assess its molecular size and charge distributions.

Application of methods **1** and **2** of Van Holde and Baldwin[6] to low-speed sedimentation-equilibrium data on *Setaria* hemicellulose B yielded values of 46,000 and 94,000 for the apparent weight-average ($\overline{M}_w^{app}$) and z-average ($\overline{M}_z^{app}$) molecular weights, respectively, of the system. These values reflect the true weight- and z-average quantities only in the event that the partial specific volumes of all species are identical and that the system exhibits ideal-solution behaviour. Of these two provisos, the latter is the more suspect, but in this respect Nichol *et al.*[7] have shown that *pronounced* non-ideality leads to the existence of a maximum in the schlieren profile: no semblance of such a phenomenon was observed in the present study, curve I of their Fig. 1 (ref. 7) typifying the equilibrium pattern obtained. Thus, although the apparent values almost certainly differ from the true average molecular-weights, the discrepancy is unlikely to be large in either instance, whereupon the values of 46,000 and 94,000 should suffice for comparative purposes: a value of 2 for $\overline{M}_z/\overline{M}_w$ is indicated.

Further information on heterogeneity with respect to molecular size has been obtained from estimation of M_z^{app} and M_w^{app} as a function of distance across the liquid column, the relevant plots being summarized in Fig. 1. The circles provide a measure of the apparent z-average molecular weight, $(M_z^{app})_r$, which is obtained[8]

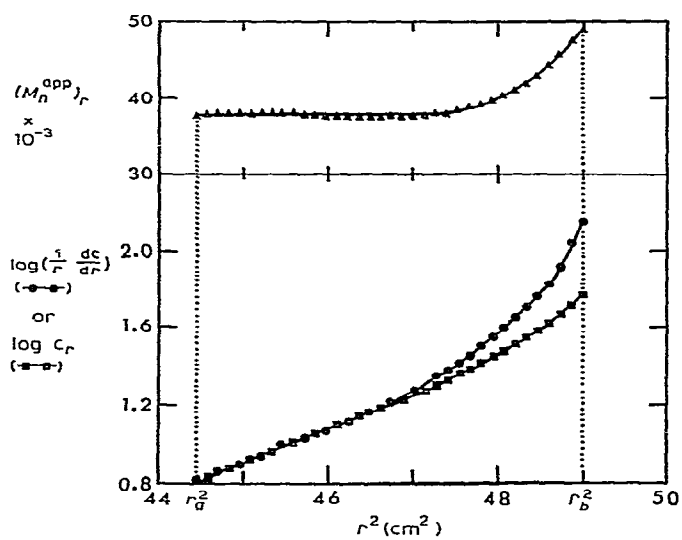

Fig. 1. Analysis of sedimentation-equilibrium patterns obtained with *Setaria* hemicellulose B in 0.05I acetate, pH 5.3. In the lower portion, squares and circles reflect the variation of the weight- and z-average molecular weights, respectively, of the system with distance in a liquid column extending from r_a to r_b. The corresponding variation in number-average molecular weight is recorded directly in the upper portion.

from the slope of the tangent to the curve at any given point r. The lower-limiting value of $(M_z^{app})_r$, obtained from the straight region of the curve, is 38,000. Squares in Fig. 1 relate to $(M_w^{app})_r$, the corresponding apparent weight-average molecular weight, which is again obtained from the tangent to the curve for any given value of r. Clearly, $(M_w^{app})_r = (M_w^{app})_r = 38{,}000$ in the vicinity of the air–liquid meniscus. This identity of apparent weight- and z-average molecular weights permits calculation of $(M_n^{app})_r$ and hence $\overline{M}_n^{app}$ for the system by the method of Lansing and Kraemer[9]. A value of 40,000 is obtained for $\overline{M}_n^{app}$, the apparent number-average molecular weight of the preparation, the variation of $(M_n^{app})_r$ with distance being summarized in the upper portion of Fig. 1. The estimate of 38,000 for $(M_n^{app})_r$, $(M_w^{app})_r$, and $(M_z^{app})_r$ throughout approximately half of the column length is typical of a system exhibiting paucidispersity rather than polydispersity with respect to molecular size. Whereas non-identity of $\overline{M}_w^{app}$ and $\overline{M}_z^{app}$ frequently reflects the extent of spread of chain lengths about a mean, the present data suggest a system composed almost entirely of polysaccharide molecules having a fairly narrow distribution of chain lengths, but also containing traces of much heavier material (for instance, 3% of solute ten times larger). Molecular aggregation of the hemicellulose[10] may well account for the presence of this heavier material.

Mention should also be made at this stage of a major discrepancy between the value of $\overline{M}_n^{app}$ found as just described and that inferred from vapour-phase osmometry of the methylated derivative: the observed $\overline{M}_n$ of 15,000 would correspond to a number-average molecular weight of 12,000 for the native hemicellulose. Although this difference between estimates could presumably reflect molecular association in aqueous environment, the argument for degradation of the polysaccharide during methylation seems more compelling. To this end it is noted that removal of heavy material from the crude hemicellulose (by preparative electrophoresis[5]) has resulted in an apparent increase in $\overline{M}_n$ from 8,000 (ref. 11) to 12,000. Thus it would appear that polysaccharides are degraded by all three methylation procedures tested previously[11].

On subjecting the purified hemicellulose to moving-boundary electrophoresis, a single, symmetrical, schlieren peak was observed (see Fig. 4 of ref. 5). However, a more definitive characterization of electrophoretic behaviour has been obtained by examining the extent of boundary spreading in terms of mobility distribution according to the method of Baldwin *et al.*[12]. In Fig. 2, which summarizes boundary analysis of descending patterns, the broken lines (– – –, · · ·, and so on) refer to apparent mobility distributions ($g^*(U)$ as a function of U) derived from exposures taken at various times during the electrophoretic experiment. Extrapolation of these $g^*(U)$ values for a given mobility to infinite time (see Fig. 3 for representative plots) leads to an estimate of $g(U)$, the true fraction of solute having mobility U: the mobility distribution obtained by this procedure is repesented by the solid line in Fig. 2. In this connexion it is noted that such correction of spread for diffusional effects would probably underestimate the true distribution in the event that polysaccharides exhibit dependence of mobility on concentration, an effect neglected in this particular application of the boundary analysis[12]. On the other hand, the mobility distribution

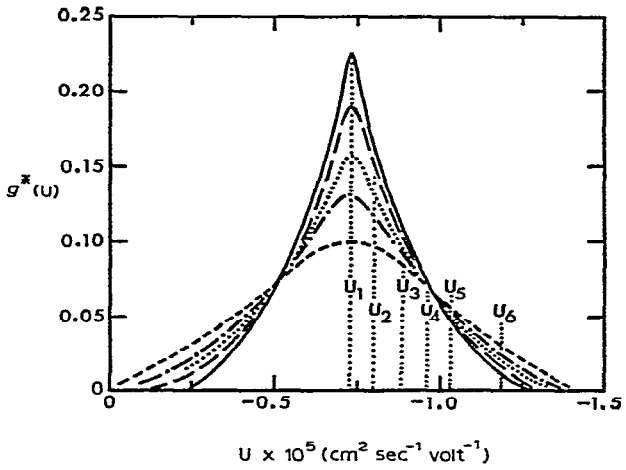

Fig. 2. Boundary analysis of descending electrophoretic patterns for hemicellulose B in 0.05I acetate, pH 5.3. ——, $t = 51$ min; –·–·–, $t = 75$ min; ·····, $t = 100$ min; – – –, $t = 172$ min. The solid line refers to the distribution obtained by extrapolation of the experimental data to infinite time (see Fig. 3).

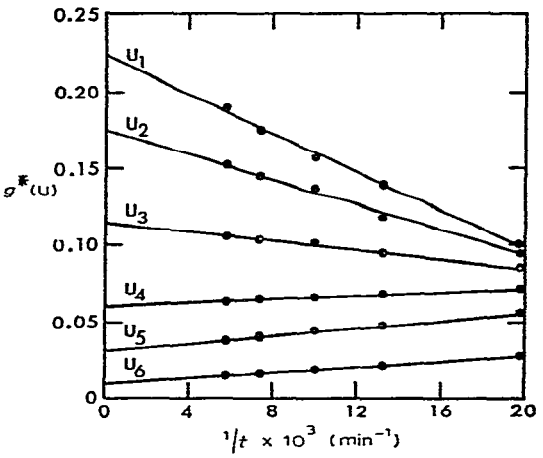

Fig. 3. Representative plots illustrating the extrapolations involved in estimating the electrophoretic polydispersity of hemicellulose B: the experimental data are taken from Fig. 2, in which the relevant mobility values (U) are indicated.

is relatively narrow, whereupon there is a distinct possibility that diffusional spreading would have approached or exceeded that due to electrophoretic polydispersity. Under these circumstances, low estimates of $g(U)$ would have been obtained in the region of the maximum of the distribution profile[13]. With the foregoing factors taken into consideration, it is concluded that Fig. 2 indicates an electrophoretic mobility of $-0.7 \pm 0.2 \times 10^{-5}$ $cm^2 sec^{-1} volt^{-1}$ for at least 80% of the purified hemicellulose.

Since electrophoretic mobility is governed largely by the ratio of charge (valence) to molecular size of a solute, the mobility distribution shown in Fig. 2 presumably reflects the heterogeneity of the hemicellulose preparation with respect to size (chain length) and/or charge. Although based on a microscopic model of electric migration that cannot be considered well established for polysaccharides, the following equation[14] provides a means of converting mobility to valence Z:

$$Z = \frac{6\pi\eta r\,(1+\kappa r+\kappa r_i)\,U}{f(\kappa r)\,(1+\kappa r_i)} \times 6.25\times10^{-9}$$

r is the radius of the solute, assumed spherical, r_i the radius of ions of the supporting electrolyte, and other quantities have their usual significance[15]. With a value of 10^{-6} cm for the average radius of hemicellulose molecules (inferred from the sedimentation coefficient[5] and molecular weight) a mobility of -0.7×10^{-5} $cm^2sec^{-1}volt^{-1}$ corresponds to a valence of -9. In this connexion it is noteworthy that chemical analysis yielded a value of 8% for the proportion of aldobiouronate in the native hemicellulose, a result that implies the existence of nine carboxylate ions per 38,000 g of polysaccharide.

In summary, this physicochemical study indicates that *Setaria* hemicellulose B is comprised mainly of arabinoxylan chains having very similar lengths and distributions of charged aldobiouronate residues. In these respects, the present results conflict with those of Blake and Richards[10], whose samples of hemicellulose exhibited not only gross heterogeneity with respect to size but also a strong tendency to aggregate. As a possible explanation of this discrepancy, we suggest the use of a milder extraction-procedure[3,11,16] in the present study. In this connexion, the extraction of the plant material with alkali prior to delignification is a departure from the usual method for preparing holocellulose. Although less hemicellulose may well have been extracted in the presence of lignin[17], the time for chlorite delignification of the crude hemicellulose preparation (30 min) was decreased considerably from that for a whole plant-extract (2–6 h), thereby minimizing the extent of degradation of the polysaccharide during this step[18,19].

EXPERIMENTAL

Isolation and purification of the polysaccharide. — Hemicellulose was isolated from *Setaria sphacelata* in accordance with the procedure described by McIlroy[16]. The resulting preparation still contained 5.9% lignin, which was removed by preparative electrophoresis on a filter-paper curtain[5]: 0.83 g of the major electrophoretic fraction was obtained from 1.95 g of the isolated polysaccharide. The purified material had $[\alpha]_D^{25}$ $-95°$ (*c* 0.25, 4% sodium hydroxide) and an ash content of 0.8%.

Methylated hemicellulose. — The sodium hydroxide–methyl sulphoxide–methyl iodide procedure (see ref. 11) was used to obtain 0.48 g of methylated derivative from 0.5 g of native hemicellulose. The resulting product had $[\alpha]_D^{25}$ $-85.3°$ (*c* 0.7, chloroform). (Found: OMe 38.8%). Vapour-phase osmometry, carried out in

the manner described previously[11], yielded an estimate of 15,000 ±700 for $\bar{M}_n$, the number-average molecular weight of the methylated hemicellulose.

Moving-boundary electrophoresis. — A solution of purified hemicellulose (0.8%) was dialysed against 0.05I acetate, pH 5.3 (2 × 500 ml) for 24 h at 4° prior to electrophoresis at the same temperature in a Perkin–Elmer model 238 electrophoresis apparatus: a current of 7.35 mA through the standard 15-mm cell was used. Enlarged tracings (×4) of schlieren patterns obtained with the phase-plate assembly were used for determining the mobility distribution (g(U) *vs.* U) by the method of Baldwin *et al.*[12], each pattern being divided into fourteen segments having a constant mobility-difference of 0.075×10^{-5} $cm^2 sec^{-1} volt^{-1}$: trapezoidal integration was used to estimate g^*(U), the proportion of total area within a segment characterized by mean mobility U. Quoted mobilities refer to migration at 0°, the temperature at which the buffer conductivity was measured according to the procedure described previously[5].

Equilibrium sedimentation. — A Spinco model E ultracentrifuge was used for estimating molecular weights of 0.4% solutions of hemicellulose dialysed against 0.05I acetate, pH 5.3. From photographic records of schlieren profiles it was found that 3-mm columns of such solutions were at sedimentation equilibrium after rotation for 22 h at 11,272 r.p.m. and 20°. Patterns were measured on a Nikon two-dimensional comparator fitted with a projection screen and accurate to 2 μm. The value of c_m, the concentration of solute at the air–liquid meniscus, was obtained (in arbitrary refractive-index units) from eqn. 5 of Richards and Schachman[20], and checked by location of the hinge point, at which the concentration equals that introduced into the cell: the corresponding value of the initial concentration was obtained by trapezoidal integration of the pattern obtained in a separate experiment employing a synthetic-boundary cell.

Pycnometry. — Densities of the 0.05I acetate buffer (pH 5.3) and of solutions containing various amounts of hemicellulose (1.0%, 0.6%, and 0.3%) were measured at 20° in a 10-ml pycnometer. The plot of density *vs.* concentration was linear over the concentration range 0–1.0% and yielded an estimate of 0.62_8 for the partial specific-volume of the polysaccharide.

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