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Interactions between locust bean gum and cellulose characterized by ^{13}C n.m.r. spectroscopy

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Abstract

Molecular interactions between locust bean gum (LBG) and cellulose crystallite surfaces appear to involve most mannosyl residues of the mannan backbone, not just the small proportion contained in long segments which lack galactosyl residues. This conclusion is based on: (1) relative strengths of ^{13}C n.m.r. signals at 102.2 ppm in the cross-polarization (CP) spectrum and 101.3 ppm in the single-pulse excitation (SPE) spectrum, assigned to mannosyl C-1 in bound and non-bound segments of LBG, respectively; (2) displacement of a ^{13}C n.m.r. signal assigned to mannosyl C-4 to 81 ppm, indicating a change of conformation in the mannan backbone relative to a gel phase; (3) similarities between proton spin relaxation time constants for cellulose and LBG, indicating proton spin diffusion between polymers in close contact; (4) broadening of CP n.m.r. signals at 83.9 and 85.0 ppm, assigned to C-4 of cellulose chains exposed on crystallite surfaces. The galactosyl C-1 signal appears at 99.9 ppm in the SPE n.m.r. spectrum of the LBG–cellulose complex but shows a poor response to CP n.m.r., indicating that galactosyl residues are not fixed in rigid conformations relative to the mannan backbones.

Keywords: Locust bean gum; Cellulose; ^{13}C n.m.r.; Galactomannan

1. Introduction

Locust bean gum (LBG) is a galactomannan with a (1 → 4)-linked β-D-mannan backbone and (1 → 6)-linked α-D-galactopyranosyl sidegroups (Dea and Morrison, 1975). It does not form strong gels on its own, but can improve the strength of gels formed by other polysaccharides, e.g., κ-carrageenan and agarose (Dea et al., 1986). It is also adsorbed on cellulose fibres, and has been used as an additive in papermaking (Swanson, 1961). Ang and Miller (1991) added powdered cellulose to a related galactomannan (guar gum) and found that the viscosity increased as an exponential function of the cellulose content, noting that this can be advantageous in food products where high viscosities are required without 'gummy' mouthfeel.

LBG is thought to have a structure that is neither regular nor statistically random (Dea et al., 1986). The weight-average length of unsubstituted mannosyl sequences is longer than expected for a statistically random distribution of galactosyl substitution. The preferred conformation of an isolated galactosyl-free mannan chain is straight and ribbon-like, with a two-fold screw axis as in the preferred conformation of cellulose (Preston, 1979). The galactosyl-free mannan sequences are therefore compatible with cellulose crystallite surfaces, though the hydrogen bonding of a mannan chain to a cellulose chain must differ from the bonding between a pair of cellulose chains on account of the conformation at C-2 of mannosyl residues (Preston, 1979).

The idea that galactosyl residues could interfere with galactomannan–cellulose interactions has not been tested by molecular modeling. Sidechains do not seem to prevent xyloglucan from binding to cellulose. Molecular modeling suggests that xyloglucan binding is initiated by a sidechain that flattens out an adjacent region of the backbone (Levy et al., 1991). Upon contacting a cellulose crystallite the flattened region spreads through successive segments. We chose to use solid-state ^{13}C n.m.r. spectroscopy to test for similar interactions between galactomannan and cellulose.

Solid-state ^{13}C n.m.r. spectroscopy has provided information about molecular interactions in galactomannan gels (Gidley et al., 1991). We used four criteria to test for molecular interactions.

(1) Relative responses to cross-polarization (CP) and single-pulse excitation (SPE) pulse sequences, which are most effective for characterizing rigid or liquid-like material, respectively.

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Table 1
Constituent sugar compositions of pulp samples (normalised mol.%)

	Glc	Xyl	Man	Gal
Untreated pulp	84.0	9.4	6.1	0.5
Pulp + LBG	66.9	6.6	22.8	3.7

(2) Changes in chemical shifts for signals assigned to the galactomannan.

(3) Changes in proton spin relaxation time constants for the LBG.

(4) Broadening of n.m.r. signals assigned to cellulose crystallite surfaces.

2. Material and methods

Locust bean gum (LBG) (Sigma Chemical Company) was described by the supplier as "believed to be a straight chain polymer of mannose with one galactose branch on every fourth mannose and a molecular weight of approximately 310 000". A sample of hydrated LBG was prepared for use as a control by adding LBG 'as received' (0.12 g) to phosphate buffer solution (3 ml, 8×10^{-3} M, pH 7.5) and heating the solution for 5 days at 100°C. The period of heating was similar to that used in the preparation of the LBG–cellulose complex (below). The product was air-dried to a thin film and later remoistened to approximately 60% moisture content.

The cellulose was a commercial sample of bleached softwood kraft pulp, supplied in the never-dried state. This particular source of cellulose was chosen because preliminary n.m.r. experiments showed a partly resolved doublet at 83.9 and 85.0 ppm, interpreted as indicating clearly defined cellulose crystallite surfaces (Newman and Hemmingson, 1995). A sample of microcrystalline cellulose was rejected because the cellulose crystallite signals were not so well resolved, making it more difficult to study the signal broadening associated with molecular interactions at crystallite surfaces.

A portion of the cellulose was treated with LBG using a procedure based on the findings of Russo and Thode (1960), i.e. that the amount of sorbed galactomannan increases with increasing time, temperature, concentration, and agitation.

Table 2
Proton spin relaxation time constants associated with ^{13}C n.m.r. signals, identified by chemical shifts rounded down to the nearest integer

		Cellulose 89 ppm	LBG 102 ppm	LBG 101 ppm
LBG alone	$T_{1\rho}$(H) (ms)	—	4.4	2.3
	T_2(H) (μs)	—	10.4	13.7
Pulp + LBG	$T_{1\rho}$(H) (ms)	33	36	—
	T_2(H) (μs)	8.2	9.6	—

Solid LBG (0.5 g) was added portionwise to phosphate buffer (50 ml, 8×10^{-3} M, pH 7.5) in a 100-ml erlenmeyer flask with stirring after each addition, giving a syrup-like solution at about 80°C. The flask was flushed with argon, placed in a bath maintained at 100°C and the contents were stirred magnetically for 30 min. Shredded pulp (2.5 g wet weight, moisture content 85%) was added portionwise to the flask taking about 10 min. The flask was then flushed with argon and held at 100°C for 81 h with continuous stirring. Excess LBG was removed in two stages. First the product was suspended twice in distilled water (100 ml) at room temperature. The suspension was stirred for 20 min and the pulp was collected on a domestic sieve. A portion of about 20% of the product was then suspended in 20 ml near-boiling distilled water, stirred for 5 min in a bath at 100°C, and collected by filtration using glass-fibre (GFC) paper. The hot-water wash was repeated twice.

The constituent sugar composition (Table 1) of commercial LBG and the LBG control sample was determined by reductive hydrolysis, acetylation and GLC analysis of the alditol acetate derivatives (Stevenson and Furneaux, 1991). For the pulp samples, hydrolysis was performed as follows: the sample (10 mg) was treated with 72% H_2SO_4 (0.125 ml) at 30°C for 2 h. The solution was then diluted to 2.25 ml, heated at 120°C for 1 h, cooled, neutralised with excess $CaCO_3$, filtered to remove the insoluble calcium salts and concentrated to dryness under reduced pressure. The dry sugars were redissolved in water (2.5 ml), refiltered and an aliquot (0.25 ml) was concentrated to dryness. The reductive hydrolysis procedure of Stevenson and Furneaux, omitting the 120°C hydrolysis step, was applied to the sugars. The resulting alditols were acetylated for GLC analysis as described above.

Samples were packed in 7-mm diameter cylindrical silicon nitride rotors and retained with Vespel end caps, using poly(chlorotrifluoroethylene) grease to form a water-tight seal. Some of the water was squeezed out during packing, leaving moisture contents in the range 50%–76% as determined after completion of n.m.r. experiments.

Rotors were spun at frequencies between 3.5 and 4 kHz in a Doty Scientific magic-angle spinning probe. Solid-state ^{13}C n.m.r. spectra were run at 50.3 MHz on a Varian Inova-200 spectrometer. Both cross-polarization (CP) and single-pulse excitation (SPE) experiments were used. In CP n.m.r. experiments, each 6-μs 90° proton preparation pulse was followed by a 1-ms cross-polarisation contact time, 30 ms of data acquisition and a recovery delay of 1 s (LBG), 3 s (pulp) or 6 s (pulp + LBG) before the sequence was repeated. These recovery delays were selected to exceed values of T_1(H) = 0.4, 1.2 and 2.1 s for the three samples, respectively. The proton decoupler power was increased during data acquisition only, to a value corresponding to a proton precession frequency of > 55 kHz. Signals were averaged over periods between 11 and 35 h for each sample.

In SPE experiments, each 6-μs 90° ^{13}C pulse was

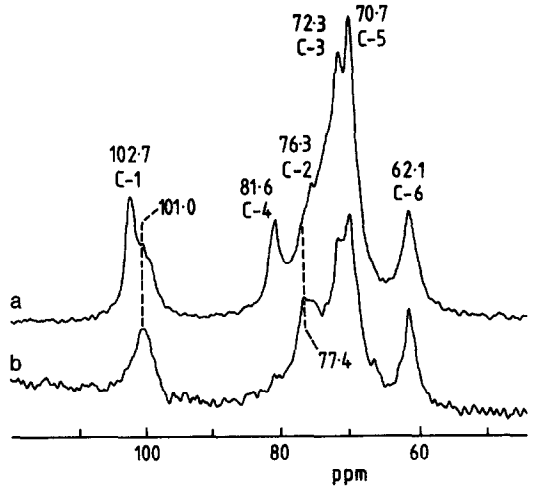

Fig. 1. ^{13}C n.m.r. spectra of the LBG control sample brought to 60% (by weight) moisture: (a) CP and (b) SPE n.m.r. pulse sequences. Carbon numbers refer to mannosyl residues. Chemical shifts are shown in ppm.

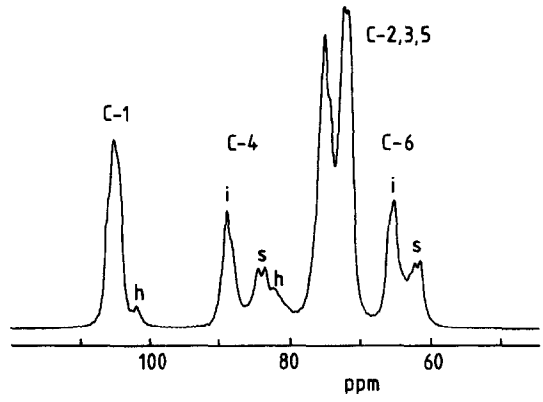

Fig. 2. ^{13}C CP n.m.r. spectra of bleached kraft pulp, part-dried to 50% moisture. Signals labelled *i* and *s* are assigned to cellulose crystallite interiors and surfaces, respectively, and signals labelled *h* are assigned to hemicelluloses. Carbon numbers refer to cellulose.

followed by 30 ms of data acquisition and a recovery delay of 1 s (LBG) or 0.5 s (pulp + LBG). Preliminary experiments confirmed that these delays were adequate for recovery of signal strength associated with the relatively mobile segments of galactomannan.

Proton rotating-frame relaxation time constants $T_{1\rho}(H)$ and spin–spin relaxation time constants $T_2(H)$ (Table 2) were determined using pulse sequences described by Alla and Lippmaa (1976).

All spectra were plotted without resolution enhancement.

3. Results and discussion

3.1. LBG control sample

Both the commercial LBG and the control sample were found to have a mannose:galactose ratio of 3.2:1. In other words, there was no evidence of hydrolysis of galactosyl residues during the heat treatment used in preparing the control sample.

The CP n.m.r. spectrum of the LBG control sample (Fig. 1*a*) shows two signals, at 102.7 and 81.6 ppm, that are not present in the SPE n.m.r. spectrum (Fig. 1*b*). The CP sequence elicits a response from solid material, and is not effective for n.m.r. of liquids, so the signals at 102.7 and 81.6 ppm are assigned to residues in relatively rigid environments. The chemical shifts of these two signals are very similar to values of 102.0 ppm (C-1) and 81.4 ppm (C-4) reported for crystalline mannan (Marchessault et al., 1990). Signals at 101.0 and 77.4 ppm in the SPE n.m.r. spectrum (Fig. 1*b*) are assigned to C-1 and C-4 of mannosyl residues in relatively liquid-like environments. The chemical shifts are similar to values of 101 and 77 ppm, respectively, reported for a gel formed from a dilute (2%, w/v) aqueous solution of LBG (Gidley et al., 1991). Signals assigned C-2, C-3, C-5 and C-6 appear at 76.3, 72.3, 70.7 and 62.1 ppm in both Fig. 1*a* and Fig. 1*b*. These chemical shifts are similar to values reported for both crystalline mannan (Marchessault et al., 1990) and a dilute LBG gel (Gidley et al., 1991), so they are not useful for distinguishing between rigid and liquid-like environments.

One of the advantages of the CP n.m.r. experiment over the SPE n.m.r. experiment is that signals are enhanced by a factor up to $\gamma_H/\gamma_C = 4.0$ provided the sample is rigid (Harris, 1983). Here γ_H and γ_C are magnetogyric ratios for protons and ^{13}C nuclei, respectively. Failure to enhance the signal at 101.0 ppm supports the assignment (above) to mannosyl C-1 in material that is not fully rigid. Comparisons of signal strengths between Fig. 1*a* and Fig. 1*b* also provide an estimate of the mannosyl residues associated with signals at 102.7 and 81.6 ppm, i.e. about 15% of all residues in the LBG.

A contribution from galactosyl C-1 is expected at a chemical shift of about 99.9 ppm (Gidley et al., 1991). This contribution appears to have been broadened to the level of an unresolved shoulder in the SPE n.m.r. spectrum (Fig. 1*b*) and possibly the CP n.m.r. spectrum (Fig. 1*a*).

The detection of relatively rigid, solid-like domains in our sample of LBG is consistent with the suggestion that at relatively high mannose:galactose ratios the protective action of galactosyl residues is insufficient to prevent crystalline association characteristic of pure mannan (Marchessault et al., 1979). The mannose:galactose ratio of 3.2:1 for LBG, reported above, refers to the overall composition. The ratio could be much higher for some segments in a structure that is neither regular nor statistically random (Dea et al., 1986). Gidley et al. (1991) did not observe any such rigid domains in a 30% w/v hydrate of LBG, but they prepared their samples at a lower temperature (40°C) than that used to prepare the sample described here (100°C). A higher preparation temperature might be needed to promote diffusion of the polymer chains to arrangements in which they can interact.

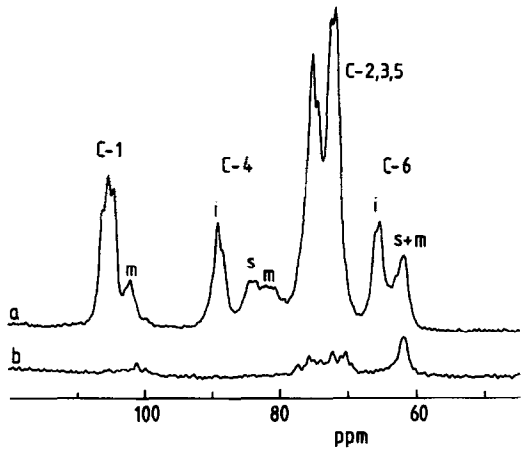

Fig. 3. ^{13}C n.m.r. spectra of bleached kraft pulp treated with LBG and part-dried to 76% moisture: (*a*) CP and (*b*) SPE n.m.r. pulse sequences. Signals labelled *i* and *s* are assigned to cellulose crystallite interiors and surfaces, respectively, and signals labelled *m* are assigned to mannosyl residues in LBG segments bound to cellulose

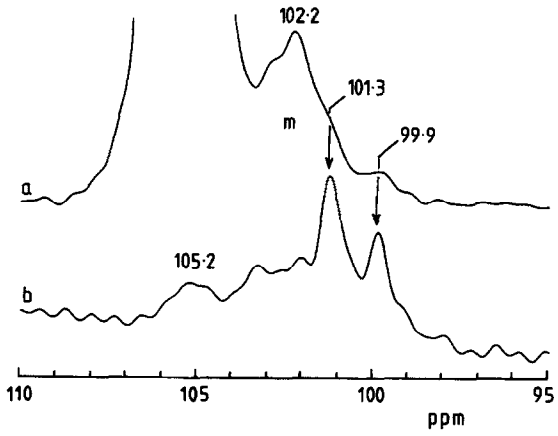

Fig. 5. The C-1 region expanded from n.m.r. spectrum of bleached kraft pulp treated with LBG and part-dried to 76% moisture: (*a*) CP n.m.r., (*b*) SPE n.m.r. Chemical shifts are shown in ppm. The vertical scale of (*b*) has been amplified by a factor of 4 relative to (*a*), in order to compensate for the theoretical enhancement of γ_H/γ_C associated with CP n.m.r.

3.2. Bleached kraft pulp

The CP n.m.r. spectrum of bleached kraft pulp (Fig. 2) is dominated by signals assigned to cellulose. A partly resolved doublet at 83.9 and 85.0 ppm, labelled *s*, is assigned to C-4 in crystallite-surface cellulose (Newman and Hemmingson, 1995). The splitting is attributed to non-equivalent sites for anhydroglucosyl units exposed at crystallite surfaces. Larsson et al. (1997) have reported similar splitting in spectra of cellulose used in their curve-fitting experiments. Their chemical-shift values of 84.1 and 83.2 ppm are offset from ours because of a difference in calibrating the chemical-shift scale.

Signals at 102.2 and 82.6 ppm, labelled *h* in Fig. 2, are assigned to hemicelluloses on grounds that they do not appear in spectra of relatively pure cellulose (Newman and Hemmingson, 1995). These signals are more clearly seen in plot expansions (Figs. 4, and 6b). The signal at 102.2 ppm is enhanced in spectra of the LBG-treated pulp (Fig. 3*a* and Fig. 4), supporting assignment to C-1 of mannosyl residues in glucomannan. Galactoglucomannans are the dominant hemicelluloses in softwoods (Timell, 1967), but the galactosyl residues are removed during kraft pulping and glucomannan chains are left adhering to the cellulose (Newman et al., 1993).

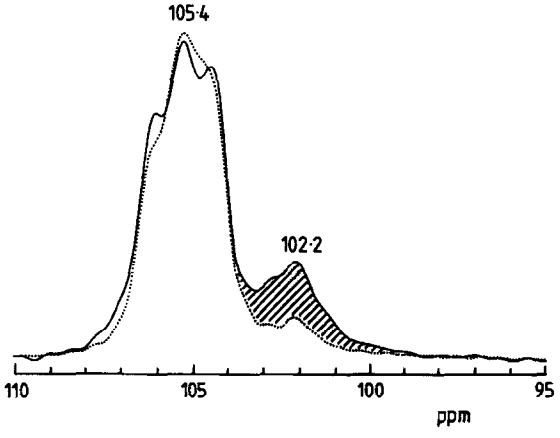

Fig. 4. The C-1 region expanded from n.m.r. spectra of (*a*) bleached kraft pulp treated with LBG, (*b*) untreated pulp. Chemical shifts are shown in ppm. The shaded area is assigned to mannosyl C-1 in LBG added to the pulp

3.3. LBG-treated bleached kraft pulp

The analytical data in Table 1 suggest that the sugar residues in this sample were distributed between those associated with the original pulp fibres (78.8%) and those added as the mannosyl and galactosyl residues of LBG (17.8 and 3.4%, respectively). The mannose:galactose ratio of 5.3:1 for the LBG in the sample of treated pulp is higher than the ratio of 3.2:1 for the original LBG. We found no evidence for hydrolysis of galactosyl residues in the LBG control sample (discussed above), so the results indicate selective binding of a fraction of the LBG with relatively low galactosyl substitution. In other words, a fraction of LBG with relatively high galactosyl substitution must have been washed from the sample.

The CP n.m.r. spectrum of the LBG-treated pulp (Fig. 3*a*) is dominated by signals assigned to cellulose, as in the spectrum of the untreated pulp (Fig. 2). Weaker signals, labelled *m* in Fig. 3, are enhanced relative to Fig. 2 and are therefore assigned to LBG. In particular, a signal at 102.2 ppm is assigned to mannosyl C-1 in well-ordered environments, i.e. segments of LBG that are sufficiently rigid to respond to CP n.m.r. The relevant chemical shift range is shown expanded in Fig. 4 (solid line) with the spectrum of the untreated pulp superimposed (dotted line). The increase in the area of the 102.2 ppm signal, relative to that for untreated pulp, corresponds to 13% of the total area for

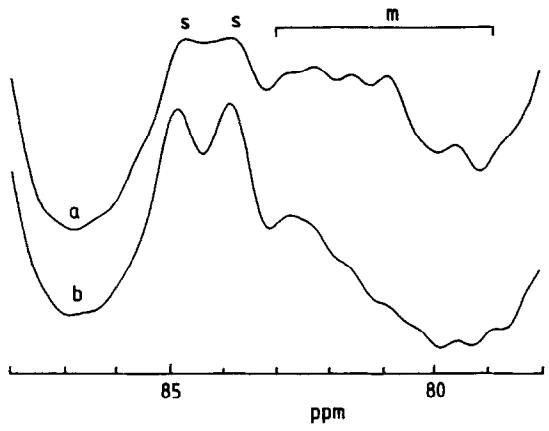

Fig. 6. The C-4 region expanded from ^{13}C n.m.r. spectra of moist samples of (*a*) bleached kraft pulp treated with LBG, (*b*) untreated pulp. Signals labelled *s* and *m* are assigned to cellulose crystallite surfaces and mannosyl residues in bound segments of LBG, respectively

the treated pulp. If the mannosyl residues of LBG account for 18% of all residues in the treated pulp, as estimated above, then it follows that about 70% of the LBG mannosyl residues respond to the CP sequence.

The SPE n.m.r. spectrum of the treated pulp (Fig. 3*b*) shows a signal at 101.3 ppm, labelled *m*, assigned to mannosyl C-1 in disordered environments. The chemical shift is very close to the value of 101.0 ppm reported (above) for the corresponding signal in n.m.r. spectra of the LBG control sample. The signal is shown more clearly in Fig. 5, in which the vertical scale of the SPE n.m.r. spectrum of the treated pulp has been amplified by a factor of 4 to compensate for the theoretical enhancement of γ_H/γ_C in the CP n.m.r. spectrum. The signal at 101.3 ppm in Fig. 5*b* overlaps adjacent signals, so the area cannot be measured with precision, but it appears to be a little less than half of the area of the signal at 102.2 ppm in Fig. 5*a*. The results are consistent with the suggestion (above) that about 70% of mannosyl residues respond to CP n.m.r.

The presence of mannosyl residues in two distinct environments, one sufficiently rigid for the residues to respond to CP n.m.r. and one sufficiently liquid-like for the residues to respond to SPE n.m.r., is consistent with a published representation of galactomannan–cellulose interactions (Marchessault et al., 1979). This distinguishes between segments adhering to the cellulose crystallite surfaces and segments forming flexible bridges between crystallites. The results are not consistent with confinement of LBG–cellulose interactions to relatively long segments of LBG lacking galactosyl residues. LBG has a distribution of galactosyl substitution that is neither regular nor completely random (Dea et al., 1986). The weight percentage of unsubstituted sequences longer than 10 mannosyl residues has been estimated as 35% for a fraction of LBG soluble in hot water, with a mannose:galactose ratio of 4.3:1 (Dea et al., 1986). This is larger than the percentage expected for a random distribution of substituents, but not large enough to account for our estimate of 70% of mannosyl residues bound in rigid environments.

The signal at 101.3 ppm in the SPE n.m.r. spectrum did not grow any stronger when the recovery delay was increased from 0.5 s (Fig. 5*b*) to 2 s (not illustrated), indicating a value of $T_1(C) \ll 0.5$ s. The signal at 102.2 ppm is partly suppressed in Fig. 5*b*, indicating a value of $T_1(C) > 0.5$ s. It is not as severely suppressed as the signal at 105.2 ppm in Fig. 5*b*, assigned to cellulose. An independent experiment (not illustrated) provided an estimate of $T_1(C) = 16$ s for C-1 of cellulose. This ranking of $T_1(C)$ values is consistent with the assignment of the signal at 102.2 ppm to semi-rigid segments of LBG, i.e. segments that are more rigid than would be expected for a disordered and hydrated polysaccharide, yet not as rigid as crystalline cellulose.

The C-1 chemical shift of 102.2 ppm is 0.5 ppm smaller than the value observed for the more rigid component of LBG alone (Fig. 1), but the difference is not large enough for an unambiguous distinction between mannan–mannan and mannan–cellulose interactions as the cause of rigidity. Other evidence favours mannan–cellulose interactions.

(1) Only about 15% of mannosyl units were contained in well-ordered chain segments in the sample of LBG control sample. The addition of cellulose was responsible for increasing the proportion to about 70%.

(2) The CP n.m.r. spectrum of LBG alone (Fig. 1*a*) shows a signal at 81.6 ppm, of similar width to the signal at 102.7 ppm. The CP n.m.r. spectrum of LBG-treated pulp (Fig. 3a) shows instead a relatively broad signal with a maximum at 81 ppm. This is labelled *m* in Fig. 3*a* and an expanded plot (Fig. 6*a*). The broadening is attributed to variations around a preferred molecular conformation. The displacement from a chemical shift of 77 ppm reported for mannosyl C-4 in a gel prepared from a 2% w/v solution of LBG (Gidley et al., 1991) indicates a preferred conformation different from that in the gel.

(3) The value of $T_{1\rho}(H)$ for protons associated with the signal at 102.2 ppm is 8 times as long as the value of $T_{1\rho}(H)$ for protons associated with the signal at 102.7 ppm in the spectrum of LBG alone (Table 2), indicating very different environments. Values of $T_{1\rho}(H)$ for protons associated with the signals at 102.2 ppm and 89.4 ppm are very similar (Table 2). The signal at 89.4 ppm is assigned to cellulose, so the similarity in $T_{1\rho}(H)$ values can be explained in terms of proton spin diffusion between cellulose crystallites and adhering LBG. In this situation, the magnetization in both domains becomes depleted at similar rates during a $T_{1\rho}(H)$ measurement (Zumbulyadis, 1983). Values of $T_2(H)$ are likewise similar for protons associated with the signal at 102.2 ppm and protons associated with cellulose (Table 2), but this parameter is less useful than $T_{1\rho}(H)$ because the range of variation between values for LBG alone and LBG-treated pulp is smaller.

(4) The doublet at 83.9 and 85.0 ppm, assigned (above) to C-4 of crystallite-surface cellulose, coalesces in the

presence of LBG (Fig. 6). This is consistent with interactions between crystallite-surface cellulose and adhering LBG, blurring the distinction between two nonequivalent crystallographic sites.

The signal at 99.9 ppm in the SPE n.m.r. spectrum (Fig. 5*b*) encloses enough area to account for all galactosyl residues in bound or non-bound segments of the LBG. Galactosyl residues contribute negligible signal strength at 99.9 ppm in the CP n.m.r. spectrum (Fig. 5*a*). This suggests that the galacatosyl residues are able to rotate around the bonds which link them to the mannan backbone, even when that backbone is bound to the cellulose crystallite surface.

4. Conclusions

The ^{13}C n.m.r. results are not consistent with confinement of LBG-cellulose interactions to relatively long segments of the mannan backbone lacking galactosyl substitution. The results indicate more generalized interactions, involving about 70% of the mannosyl residues but not the galactosyl residues.

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