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Selective removal of α-D-galactose side chains from *Rhizobium* capsular polysaccharide by guar α-D-galactosidase: effect on conformational stability and gelation

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ABSTRACT

α-D-Galactosidase, isolated from germinating seeds of guar, removed up to 48% of the (1 → 2)-linked α-D-galactose side chains of *Rhizobium* capsular polysaccharide (CPS), with no loss of the disaccharide side chains (1 → 6)-linked to the same backbone residues in the hexasaccharide repeating units. Results from differential scanning calorimetry (DSC) and measurements of gel rigidity (G') indicate that removal of α-D-galactose side chains facilitates adoption of the "pseudo double-helical" structure proposed from X-ray fibre diffraction analysis (increase in T_m and reduction in DSC peak width for conformational ordering on cooling), but progressively eliminates the helix–helix aggregation necessary for gel formation (reduction in thermal hysteresis and in G').

INTRODUCTION

Irregularly spaced side chains on otherwise linear polysaccharides promote conformational disorder and solubility by inhibiting ordered assembly in the solid state and by enhancing the conformational entropy of the disordered coil form in solution. The best characterised example is the galactomannan family of energy-reserve polysaccharides from legume seeds, which are comprised of (1 → 4)-β-D-mannans with different degrees and patterns of substitution by 6-linked side chains of single α-D-galactose residues[1]. The unsubstituted mannan is insoluble in water; increase in the content of α-D-galactose increases the ease of dissolution and extent of solubility. Regions of unsubstituted mannan residues appear to be critical for self association and in gelling interactions with other polysaccharides[2–4]. Indeed, a commercially viable procedure has been reported for enhancing the

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functional interactions of a cheap and readily available galactomannan, guar gum, by controlled debranching with α-D-galactosidase[5].

However, regularly spaced side chains in bacterial polysaccharides can have the opposite effect, of promoting conformational order, by incorporation as an integral part of the ordered structure. The first illustration of such behaviour was in xanthan (the exopolysaccharide from *Xanthomonas campestris*), where charged trisaccharide side chains attached to alternate residues in the cellulosic backbone drive the formation of a 5_1 ordered structure rather than the normal 2_1 conformation of unsubstituted cellulose[6]. More recently, it has been demonstrated[7] that the side chains in welan and rhamsan (mono-[8] and di-saccharides[9], respectively) substantially increase the thermal stability of the ordered structure[10] compared to that of the unbranched parent polysaccharide (gellan).

Regularly spaced side chains also have a crucial role in the conformation and functional interactions of the capsular polysaccharide (CPS) produced[11] by many strains of *R. trifolii* and *R. leguminosarum*. *Rhizobium* CPS resembles agar in forming stiff, turbid gels at low concentrations[11,12], with substantial thermal hysteresis between the transition-midpoint temperatures (T_m) for the formation and melting of the underlying ordered structure[12]. The backbone has[11,12] the trisaccharide repeating sequence → 4)-α-D-Glc-(1 → 3)-α-D-Man-(1 → 3)-β-D-Gal-(1 → with the side chains β-D-Gal-(1 → 4)-β-D-Gal and α-D-Gal attached to the α-D-Glc residue at positions 6 and 2, respectively. Oxidation of the side chains (yielding charged carboxylic derivatives) progressively reduces the T_m values[13]. Complete removal of the side chains by Smith degradation abolishes conformational ordering and gel formation[12]. These indications that the side chains form a necessary, integral part of the ordered structure are reinforced by a detailed analysis of X-ray fibre diffraction patterns[14], with the best fit between observed and calculated intensities coming from a single-helix model in which the side chains are draped around the main chain to give a "pseudo double helix".

In the present work, we have explored the role of the individual side chains, by using guar α-D-galactosidase to remove a proportion of the α-D-galactose side chains whilst leaving the β-linked disaccharide side chains intact.

EXPERIMENTAL

CPS from *Rhizobium trifolii* (strain TA-1) was kindly donated by Dr. L.P.T.M. Zevenhuizen (Laboratory of Microbiology, Agricultural University, Wageningen, The Netherlands) and was identical to the sample used in the investigation of structure and gelation[12]. α-D-Galactosidase was purified[15] from germinating seeds of guar. For initial evaluation of enzyme activity, a solution of the CPS (30 mg) in deionised water (30 mL) was prepared at 90° and cooled to 45°; 2 M NaOAc buffer (pH 4.5, 0.25 mL) was added, followed by 11 U of enzyme (0.1 mL of a preparation of 110 U/mL), and the solution was incubated at 45°. Samples were withdrawn after 30, 60, and 130 min, then immediately heated to 90–95° for 10 min to

TABLE I

Evaluation of the action of α-D-galactosidase on *Rhizobium* CPS

Incubation temperature (°)	45	45	45	50	50	50
Incubation time (min)	30	60	130	30	60	130
α-D-Galactose released (%)	2.3	6.1	7.1	2.0	4.9	4.7
Enzyme activity [a] (nmol/min/U)	2.1	2.7	1.5	1.8	2.2	1.0

[a] Enzyme activity against *p*-nitrophenyl α-D-galactopyranoside is defined as 1000; typical initial activities against guar galactomannan were in the range 4000–5000.

inactivate the enzyme, and the amount of galactose liberated (Table I) was determined by the galactose dehydrogenase–NADH assay[5]. The experiment was repeated at 50°, and comparative measurements were made using guar galactomannan or *p*-nitrophenyl α-D-galactopyranoside as substrate.

Samples with various degrees of monosaccharide depletion were prepared by incubation of CPS (50 mg) at 45° for different times and/or with different amounts of α-D-galactosidase (Table II). After heating to inactivate the enzyme (as before), each product was dialysed thrice against deionised water and freeze-dried. The degree of debranching was determined by ^{1}H-NMR spectroscopy at 200 MHz (Bruker AM 200 instrument), using a 10-s delay between consecutive 90° pulses in order to ensure complete relaxation.

Conformational transitions on heating and cooling were monitored by differential scanning calorimetry (DSC) with a Setaram microcalorimeter, using scan rates of 0.2, 0.5, and 1.0 deg/min, at a polysaccharide concentration of 10 mg/mL in

TABLE II

Preparation, composition, and enthalpy values of *Rhizobium* CPS samples

CPS sample	A	B	C	D	E	F
α-D-Galactose removal (%)	0	11	20	25	37	48
Incubation time (min) [a]	0	20	60	120	240	1200
Enzyme units	0	175	150	175	150	700
Enzyme activity (nmol/min/U) [b]		1.6	1.1	0.6	0.5	0.003
Average molecular weight per repeat unit	973	953	937	928	906	887
ΔH (J/g) [c]						
heating	19.4	21.4	19.5	21.4	17.6	17.1
cooling	19.3	22.4	20.1	21.0	17.4	17.3
ΔH (kJ/mol)						
mean (6 scans)	19.9	23.0	21.1	22.8	19.3	19.4
standard deviation	0.2	0.4	1.4	0.7	2.3	0.6

[a] Incubation was at 45°, using 50 mg of polysaccharide at a concentration of 1 mg/mL. The extent of removal of α-D-galactose was determined by ^{1}H-NMR spectroscopy of the modified polysaccharides. [b] Enzyme activity against *p*-nitrophenyl α-D-galactopyranoside is defined as 1000. [c] ΔH values are means of 3 heating scans and 3 cooling scans (at 0.2, 0.5, and 1.0 deg/min) at a polysaccharide concentration of 10 mg/mL.

deionised water. Gel rigidity (storage modulus, G'), also at 10 mg/mL, was measured at 10° with a Sangamo Viscoelastic Analyser at 2.5 rad/s, using cone-and-plate geometry of cone angle 2° and diameter 5 cm.

RESULTS

Action of α-D-galactosidase on Rhizobium *CPS.*—Table I shows the results of a preliminary evaluation of the action of guar α-D-galactosidase on *Rhizobium* CPS at 45°, the minimum practical temperature to avoid gelation of the polysaccharide. Although the α-D-galactose side chains on CPS are (1 → 2)-linked to α-D-glucose, rather than (1 → 6)-linked to β-D-mannose as in the normal galactomannan substrate, significant release of galactose was observed, but the specific activity of the enzyme was 3 orders of magnitude lower than when guar galactomannan or *p*-nitrophenyl α-D-galactopyranoside was used as substrate. The activity was reduced (Table I) on raising the temperature to 50° and on prolonged incubation, consistent with the known[15] instability of the enzyme above 40°. The maximum degree of debranching achieved in these preliminary experiments was just over 7%. Samples with up to 48% of the α-D-galactose side chains removed (Table II) were prepared by using higher concentrations of enzyme and longer incubation times.

Analysis of CPS samples by ^{1}H-NMR spectroscopy.—Fig. 1 shows illustrative spectra for the H-1 resonance of *Rhizobium* CPS before and after removal of a substantial proportion (37%) of the α-D-galactose side chains. In both spectra, the resonances below ~ 4.7 ppm come[12] from β-D-galactose residues (two in the disaccharide side chain and one in the main chain for each hexasaccharide repeating unit), and their integrated intensity provides a convenient, invariant reference. The peak at 5.03 ppm is from α-D-mannose. The remaining two signals (centered at 5.17 and 5.40 ppm) in the spectrum of the unmodified CPS are assigned, respectively, to the α-D-galactose side chain and the α-D-glucose residue to which it is attached[12]. In the spectrum of the partially debranched CPS, the intensity of the signal at 5.17 ppm is reduced, as expected. The intensity of the signal at 5.40 ppm, from doubly branched α-D-glucose, is also reduced, and a new signal is present at ~ 5.21 ppm, attributed to α-D-glucose residues that no longer carry a side chain at position 2. The assignment is consistent with the downfield shift of ~ 0.2 ppm in the H-1 signal that normally accompanies 2-substitution[16].

The signals at 5.17 (from α-D-galactose side chains) and 5.21 ppm (from singly branched α-D-glucose) overlap, and therefore cannot be reliably resolved during integration. However, because these resonances arise, respectively, from repeating units with and without a monosaccharide substituent, they should be present invariably in exactly complementary amounts, giving a combined integral corresponding to one anomeric proton (i.e., in the ratio 1 : 1 : 3 in comparison with the signals from α-D-mannose and β-D-galactose). The observed intensities for all preparations were in acceptable agreement with this expectation.

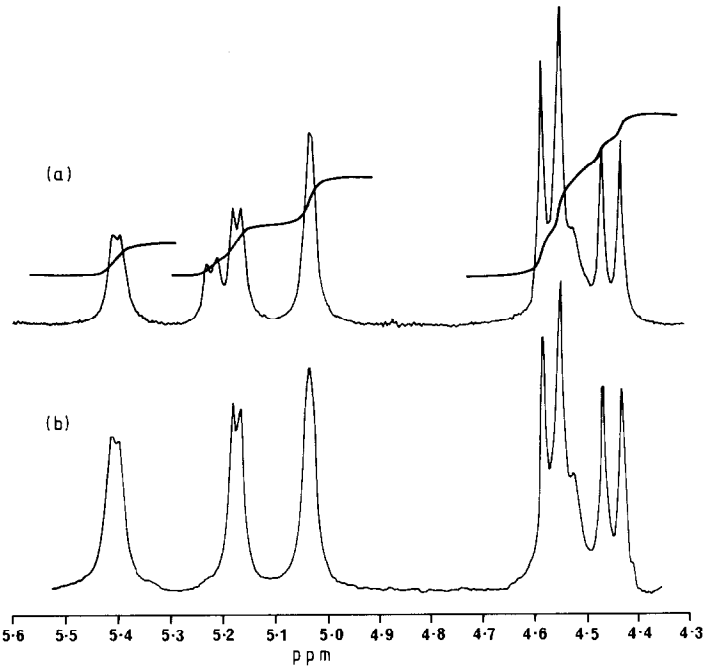

Fig. 1. ^{1}H-NMR spectra (90°, 10 mg/mL in D_2O) in the H-1 region for (a) sample E (37% of α-D-galactose side chains removed) and (b) *Rhizobium* CPS (Sample A). The integrals used to quantify the loss of α-D-galactose are shown in (a).

Since there is also slight overlap between the α-D-galactose signal at 5.17 ppm and the α-D-mannose resonance at 5.03 ppm, the precision of the analysis was improved further by including the latter signal in the combined integral and adjusting the limits of integration to give an accurate 2:3 ratio with the intensity of the signal from β-D-galactose. These integrals (over the approximate ranges 5.3 to 4.9 and 4.8 to 4.3 ppm, respectively) were then used to determine the degree of debranching (Table II), by comparison with the intensity of the signal at 5.40 ppm from residual doubly branched α-D-glucose.

Effect of debranching on gel formation.—Six samples were used for physical studies of gel formation and conformational change, namely, unmodified CPS (A) and samples with 11 (B), 20 (C), 25 (D), 37 (E), and 48% (F) of the α-D-galactose side chains removed. Sample F was non-gelling; the other samples formed gels on cooling, but with a progressive decrease in rigidity (G') from A to E (Fig. 2). However, the obvious interpretation that removal of side chains causes progressive loss of the ordered structure necessary for network formation is incorrect, as demonstrated below.

Differential scanning calorimetry.—A puzzling feature of the behaviour of unmodified CPS is that the changes in optical rotation observed in the initial stages of gel melting are often in the same direction as the simple sigmoidal process

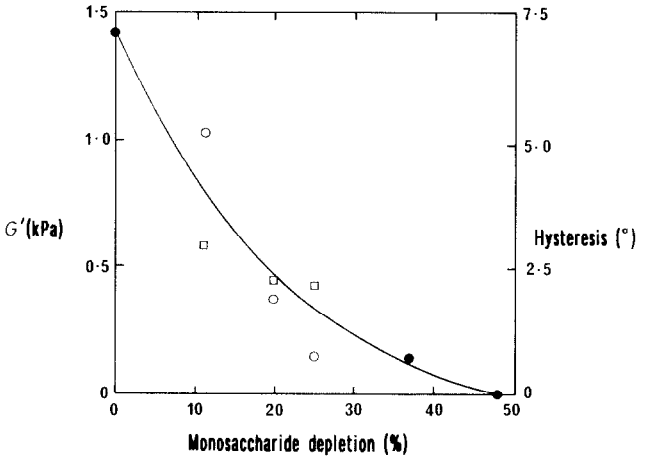

Fig. 2. Effect of removal of the α-D-galactose side chains from *Rhizobium* CPS on gel rigidity (□, storage modulus, G') and on thermal hysteresis between formation and melting of the ordered structure (○); ●, co-incident values.

accompanying gel formation on cooling, with the expected reversal of direction occurring only at higher temperature[12]. In studies that will be reported in detail elsewhere, we have confirmed a suggestion[17] that this anomalous behaviour in optical rotation arises from chiral scattering by supramolecular assemblies, and shown that it has no counterpart in the thermal changes detected by DSC. Thus, DSC is the preferable technique for following conformational transitions of *Rhizobium* CPS, without complication from higher levels of structural organisation.

Fig. 3 shows illustrative DSC traces for partially debranched CPS (sample D) on heating and cooling at scan rates of 0.2, 0.5, and 1.0 deg/min. There is an "overshoot" effect in each direction, with peaks at higher scan rates being progressively displaced in the direction of temperature change. The apparent T_m values, however, vary linearly with scan rate (Fig. 4) and can be extrapolated to the true T_m at zero rate. The values obtained in this way for the CPS (sample A) agree closely with previous results from optical rotation measurements on the same sample[12], with T_m for melting of the ordered structure exceeding T_m for conformational ordering on cooling by $\sim 7.5°$.

As illustrated in Fig. 4, the thermal hysteresis is decreased by removal of a proportion of the α-D-galactose side chains. Within the scatter of experimental results, the decrease in hysteresis and the accompanying decrease in gel rigidity have approximately the same dependence on the degree of debranching (Fig. 2), consistent with the suggestion[12] that, as in other polysaccharide systems[18], thermal hysteresis and gel formation have a common origin in helix–helix aggregation. At the highest degree of debranching achieved (48%, sample F), hysteresis and gelation were undetectable.

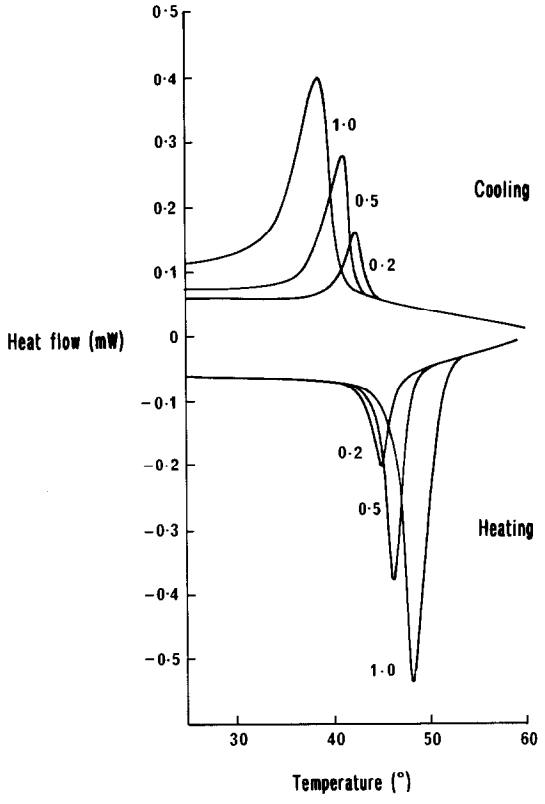

Fig. 3. DSC of partially debranched *Rhizobium* CPS (10 mg in 1 mL), illustrated for sample D (25% of α-D-galactose side chains removed). The scan rates (deg/min) used are shown beside the corresponding peaks.

However, removal of the α-D-galactose side chains was accompanied by a progressive increase (Fig. 5) in T_m for formation of the ordered structure on cooling (from ~41° for sample A to ~48° for sample F). Because of the associated decrease in thermal hysteresis (Fig. 2), the T_m values for the order–disorder transition on heating decrease (Fig. 5) with increasing degree of debranching for samples A (0%) to D (25%) and then increase at higher extents of debranching, as the hysteresis drops to zero.

As expected, the changes in enthalpy (ΔH values, Table II) for each sample are the same, to within experimental error, on heating and cooling, and have no systematic dependence on scan rate (the reduction in peak size with decreasing scan rate in Fig. 3 is due simply to lower rates of change in temperature giving a proportionate reduction in heat flow for the same overall change in enthalpy). The ΔH value per unit weight of polysaccharide for sample F is slightly lower than for sample A, but, when expressed on a molar basis (taking the mean molecular weight per backbone repeat), they become virtually identical. The molar ΔH values for

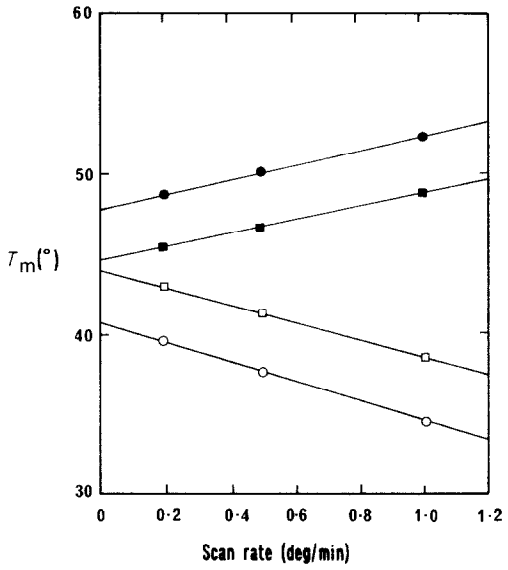

Fig. 4. Scan-rate dependence of apparent T_m values from DSC, illustrated for samples A (○, ●) and D (□, ■) on heating (●, ■) and cooling (○, □). The true T_m values are obtained by linear extrapolation to zero scan rate, as shown.

samples with intermediate degrees of debranching (B–D) are slightly higher than those at either extreme, but the differences are scarcely beyond the scatter of experimental results. Overall, there is no obvious systematic change in ΔH with progressive removal of the α-D-galactose side chains up to 48%.

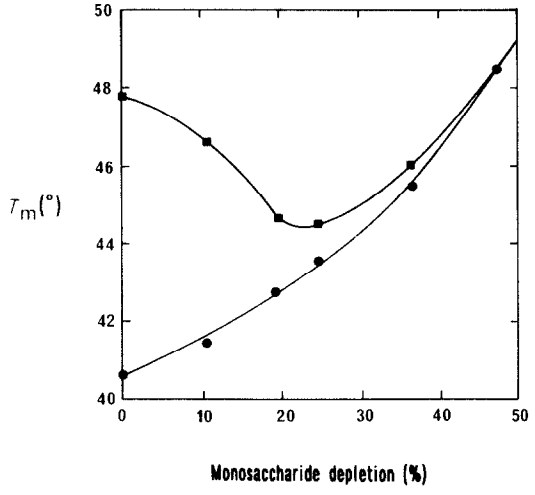

Fig. 5. Variation of T_m values from DSC for formation (●) and melting (■) of the ordered structure of *Rhizobium* CPS (10 mg/mL) on progressive removal of the α-D-galactose side chains.

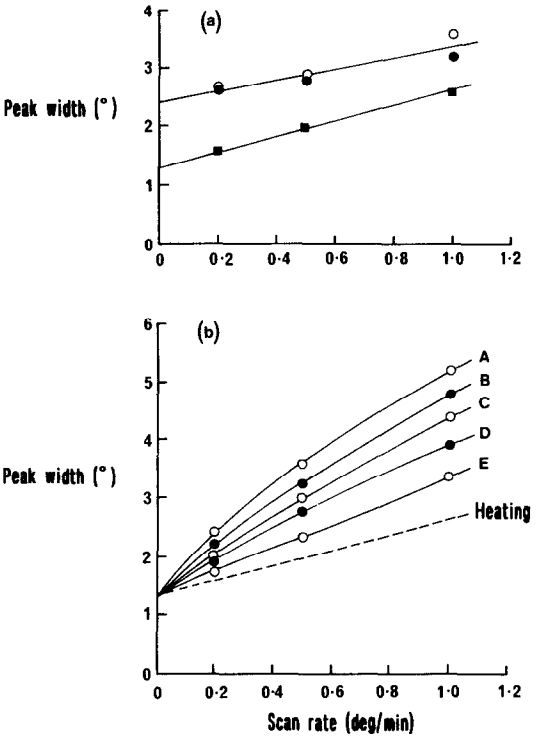

Fig. 6. (a) Scan-rate dependence of peak width in DSC (measured at half maximum height) for non-gelling sample F on heating (●) and cooling (○). Heating peaks for gelling samples A–E have the same widths, with the linear scan-rate dependence shown (■). (b) Scan-rate dependence of peak width for samples A–E on cooling; the widths of the heating peaks for the same samples [■ in (a)] are shown again for direct comparison (– – –).

The peak widths in DSC for the order–disorder (heating) transition are the same, to within experimental error, for all five gelling samples (A–E), and the mean values decrease linearly with decreasing scan rate (Fig. 6a). The corresponding cooling peaks (disorder–order transition) are substantially wider. The difference in peak width between the cooling and heating scans decreases with decreasing scan rate and with increasing degree of debranching (Fig. 6b). For the non-gelling sample F (48% removal of α-D-galactose side chains), the DSC peaks on heating and cooling are virtually identical in width at equivalent scan rates. They are systematically wider (Fig. 6a) than the heating endotherms for the other samples, but show approximately the same slope of scan-rate dependence.

DISCUSSION AND CONCLUSIONS

At the outset of this investigation, it was not obvious that guar α-D-galactosidase would remove α-D-galactose side chains from *Rhizobium* CPS, since they are

2-linked to α-D-glucose, whereas, in the usual galactomannan substrates, they are 6-linked to α-D-mannose. Although the removal was incomplete, the extent of debranching achieved (up to 48%) was sufficient to give useful insight into the effect of the α-D-galactose side chains on conformational ordering and intermolecular association.

Conformational stability and aggregation.—The most unexpected result was the observed increase in T_m for conformational ordering on progressive debranching, showing that removal of a proportion of the α-D-galactose side chains facilitates adoption of the ordered structure. From standard thermodynamic relationships:

$$\Delta G = \Delta H - T\Delta S = 0 \text{ at } T_m; \quad T_m = \Delta H/\Delta S$$

Since there is no systematic increase in ΔH with degree of debranching across the range studied, the increase in T_m is probably due to a reduction in ΔS, although the changes are sufficiently small (from $T_m \approx 314$ K for sample A to $T_m \approx 321$ K for Sample F) that they could arise from a slight upward trend in ΔH masked by the scatter of experimental results (Table II). The simplest interpretation, however, is that the α-D-galactose side chains contribute little to the enthalpic stability of the ordered structure, and that the dominant effect of their removal is to decrease the loss of conformational entropy on conversion from the disordered into the ordered state (by having fewer residues to immobilise). The sharp decrease in thermal hysteresis and gel rigidity with increasing degree of debranching, by contrast, strongly indicates that the α-D-galactose side chains have a crucial role in network cross-linking by helix–helix aggregation.

Kinetics of conformational ordering.—Another unexpected feature of the experimental results was the wide variation in the width of the DSC peaks. On melting (which is normally an equilibrium process[18]), peak widths for all the gelling samples were the same, and the variation with increasing scan rate was restricted to the small, linear increase expected from thermal lag due to finite rates of heat transfer within the calorimeter cell. The much wider peaks and greater scan-rate dependence in the cooling direction suggest that the kinetics of the disorder–order transition are unusually slow, on the same time scale as the rate of temperature change in the calorimeter (i.e., s–min in comparison with ms–s for other polysaccharides such as carrageenan[19] or xanthan[20]).

The most likely restriction on the rate of conformational ordering is the extremely crowded steric environment around the doubly branched backbone residue. This interpretation is consistent with the systematic reduction in peak-width and scan-rate dependence in DSC on progressive removal of α-D-galactose side chains. By 48% removal (sample F), the scan-rate dependence in the cooling direction is virtually the same as that on heating (i.e., probably arising almost entirely from simple thermal lag), but the melting endotherms are substantially wider than those of the other samples, indicating a reduction in co-operativity at this degree of debranching.

Comparison with the "pseudo double-helical" model from X-ray studies.—The fibre diffraction patterns so far obtained for *Rhizobium* CPS are from oriented, non-crystalline specimens, and are therefore lacking in Bragg reflections. With modern techniques of computer modelling and structural refinement, however, it has been possible[14] to identify several stereochemically feasible models and to select the most likely of these by the criteria of agreement with observed layer-line intensities (reliability index, R) and their ability to pack within the unit cell. Five candidate structures with acceptable R values were generated, namely, two double-helix models and three single helices. The double-stranded structures were rejected because they could not be packed together without severe steric compression. The best-fitting single-helix structure (i.e., lowest R factor) was further refined to yield the final "pseudo double-helical" model.

In the optimum packing arrangement for this structure, the hydrogen-bonding contacts between contiguous helices all involve side-chain residues, consistent with the experimental evidence of a progressive reduction in helix–helix aggregation as the α-D-galactose side chains are removed. The accompanying increase in T_m for adoption of the ordered structure, and the constancy of ΔH, however, are more difficult to reconcile with the proposed model.

In the "pseudo double-helix" structure, each α-D-galactose side chain is hydrogen bonded to the adjacent α-D-glucose residue in the main chain and to the terminal β-D-galactose of the disaccharide side chain on the next repeating unit in the reducing direction. The disaccharide side chains, although stiffened by internal hydrogen bonds between the two residues, have no direct bonding contact with the main chain. Therefore, removal of α-D-galactose side chains would be expected to destroy the "pseudo double helix", whereas it is clear from the above results that the ordered structure survives, and indeed is stabilised.

An interpretation that may reconcile this apparent conflict of evidence is that the "pseudo double helix" is invariably stabilised by non-coaxial side-by-side associations of the type postulated[14] from computer modelling. The turbidity of *Rhizobium* CPS gels, and the substantial thermal hysteresis between their formation and melting, argue strongly for extensive helix–helix aggregation. The disorder–order transition, however, occurs as a single, sharp process, whether monitored by DSC or by optical rotation[12]. It is feasible, therefore, that although the fundamental structural unit is an intramolecular single helix, its formation occurs as an intermolecular process, with mutual stabilisation of two or more growing helices. A mechanism of this type might explain both the low rate of conformational ordering and the unusual sharpness of the disorder–order and order–disorder transitions.

The formation and association of two independent helix nuclei, with immobilisation of their pendant side chains, might reasonably be expected to be a slow process. After successful nucleation in two associated chains, however, subsequent growth, driven by simultaneous aggregation, might be comparatively rapid, leading

to extended helical structures with, therefore, highly co-operative formation and melting, as observed.

Slow nucleation and rapid growth would also be expected to favour addition of extra chains to existing aggregates, in preference to initiation of new ordered structures, with consequent further increase in co-operativity. It may therefore be significant that in the most extensively de-branched sample (F), where the extent of aggregation was insufficient to cause detectable thermal hysteresis or network formation, the co-operativity of the order–disorder transition, as indicated by peak width in DSC, was also lower than in the other samples.

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REFERENCES

1 I.C.M. Dea and A. Morrison, *Adv. Carbohydr. Chem. Biochem.*, 31 (1975) 241–312.
2 I.C.M. Dea, A.H. Clark, and B.V. McCleary, *Carbohydr. Res.*, 147 (1986) 275–294.
3 I.C.M. Dea, A.H. Clark, and B.V. McCleary, *Food Hydrocolloids*, 1 (1986) 129–140.
4 I.C.M. Dea and D.A. Rees, *Carbohydr. Polym.*, 7 (1987) 183–224.
5 P.V. Bulpin, M.J. Gidley, R. Jeffcoat, and D.R. Underwood, *Carbohydr. Polym.*, 12 (1990) 155–168.
6 R. Moorhouse, M.D. Walkinshaw, and S. Arnott, *ACS Symp. Ser.*, 45 (1977) 90–102.
7 G. Robinson, C.E. Manning, and E.R. Morris, in E. Dickinson (Ed.), *Food Polymers, Gels and Colloids*, RSC Special Publication No. 82, Cambridge, 1991, pp. 22–33.
8 M.A. O'Neil, R.R. Selvendran, V.J. Morris, and J. Eagles, *Carbohydr. Res.*, 147 (1986) 295–313.
9 P.-E. Jansson, B. Lindberg, J. Lindberg, and E. Maekawa, *Carbohydr. Res.*, 156 (1986) 157–163.
10 R. Chandrasekaran, L.C. Puigjaner, K.L. Joyce, and S. Arnott, *Carbohydr. Res.*, 181 (1988) 23–40.
11 L.P.T.M. Zevenhuizen and A.R.W. van Neerven, *Carbohydr. Res.*, 124 (1983) 166–171.
12 M.J. Gidley, I.C.M. Dea, G. Eggleston, and E.R. Morris, *Carbohydr. Res.*, 160 (1987) 381–396.
13 A. Cesaro, P. Esposito, C. Bertocchi, and V. Crescenzi, *Carbohydr. Res.*, 186 (1989) 141–155.
14 E.J. Lee and R. Chandrasekaran, *Carbohydr. Res.*, 231 (1992) 171–183.
15 B.V. McCleary, *Phytochemistry*, 22 (1983) 649–658.
16 T. Usui, M. Yokoyama, N. Yamaoka, K. Matsuda, K. Tuzimura, H. Sugiyama, and S. Seto, *Carbohydr. Res.*, 33 (1974) 105–116.
17 A. Cesaro, S. Paoletti, F. Delben, S. Cavallo, V. Crescenzi, and L.P.T.M. Zevenhuizen, in S.S. Stivala, V. Crescenzi, and I.C.M. Dea (Eds.), *Industrial Polysaccharides*, Gordon and Breach, New York, 1987, pp. 99–109.
18 E.R. Morris and I.T. Norton, in E. Wyn-Jones and J. Gormally (Eds.), *Aggregation Processes in Solution*, Elsevier, Amsterdam, 1983, pp. 549–593.
19 I.T. Norton, D.M. Goodall, E.R. Morris, and D.A. Rees, *J. Chem. Soc., Faraday Trans. 1*, 79 (1983) 2489–2500 and 2501–2515.
20 I.T. Norton, D.M. Goodall, S.A. Frangou, E.R. Morris, and D.A. Rees, *J. Mol. Biol.*, 175 (1984) 371–394.