ELSEVIER

PII: S0144-8617(96)00059-8

Carbohydrate Polymers 30 (1996) 165–175

0144-8617/96/$15.00 + .00

Conformational and rheological transitions of welan, rhamsan and acylated gellan

E.R. Morris,* M.G.E. Gothard,[1] M.W.N. Hember, C.E. Manning[2] & G. Robinson[3]

Department of Food Research & Technology, Silsoe College, Cranfield University, Silsoe, Bedford, MK45 4DT, UK

Native ('high acyl') gellan adopts double helix geometry at a much higher temperature than the deacylated polymer (commercial gellan gum), but the resulting gels are weaker, more elastic, and show no thermal hysteresis between formation and melting, indicating that acetyl groups, which are located on the periphery of the helix, prevent aggregation. On progressive removal of glyceryl substituents, which are located in the core of the helix and modify its geometry, the disorder–order transition becomes broader (i.e. less co-operative) and moves to a lower temperature. Eventually a second transition appears at the position characteristic of the fully deacylated polymer. Comparison of the relative magnitudes of the two transitions with the proportion of residual glycerate indicates that conversion from 'high acyl' to 'deacetylated' geometry requires six consecutive repeating units devoid of glyceryl groups.

In welan and rhamsan, the double helix is stabilised to temperatures above 100°C by incorporation of, respectively, monosaccharide and disaccharide sidechains in the ordered structure. Both have 'weak gel' properties similar to those of xanthan. However, 'true' gels are formed when the helix structure is dissociated and regenerated (by dissolving welan in dimethyl sulphoxide and adding water, or by heating and cooling deacylated rhamsan in aqueous solution). Our interpretation of this behaviour is that the native structures of both polymers are perfect double helices, with exact pairing of strands along the full length of the participating chains. Dissociation of these 'perfect' structures allows development of a cross-linked network by individual chains forming shorter helices with more than one partner. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The physical properties of polysaccharides can be altered radically by the presence of sugar side-chains or non-carbohydrate appendages. For example, chemical derivatisation of cellulose is used extensively to convert the polymer from its insoluble native state into soluble materials with wide-ranging industrial applications (Whistler & BeMiller, 1993). In some cases, solubility is conferred by the introduction of charged groups, which destabilise the fibrillar structure by electrostatic repulsion. More generally, however, chemical substituents may promote solubility by presenting a physical barrier to close-packing of the polysaccharide chains, and by contributing to conformational entropy in the solution state.

*Corresponding author.
[1]Unilever Research, Colworth Laboratory, Sharnbrook, Bedford, MK44 1LQ, UK.
[2]Present address: Institute of Food Research, Norwich Research Park, Colney, Norwich, NR4 7UA, UK.
[3]Present address: Baker Hughes Inteq, P.O. Box 670968, Houston, TX 77267-0968, USA

Naturally-occurring substituents can, of course, act in the same way. For example, konjac glucomannan (Nishinari *et al.*, 1992) which comprises β-D-glucose and β-D-mannose residues linked (1→4)-diequatorially (as in cellulose), is solubilised by a very low level of acetate substitution (about 1 acetyl group per 17 sugars). Removal of these substituents (by exposure to alkali) allows the polymer chains to associate and form a gel network. In galactomannans, such as locust bean gum, tara gum and guar gum, solubility is conferred by irregularly-spaced single-sugar sidechains of α-D-galactose linked (1→6) to a proportion of the mannose residues of the (otherwise insoluble) mannan backbone (Dea & Morrison, 1975).

Regularly-spaced carbohydrate side-chains, such as occur in many microbial polysaccharides, can have a more specific role, by packing along the polymer backbone to form an integral part of a regular, ordered structure. The best known example is the 5-fold conformation of xanthan (Moorhouse *et al.*, 1977), the exopolysaccharide from *Xanthomonas campestris* (Jeanes *et al.*, 1961). Xanthan has a cellulosic backbone

with charged trisaccharide side-chains attached to alternate glucose residues, to give a pentasaccharide repeating unit (Jansson *et al.*, 1975; Melton *et al.*, 1976). At high temperature and low ionic strength, it exists in solution as a disordered coil, but converts to the 5-fold helix structure on cooling and/or addition of salt (Morris, 1973; Holzwarth, 1976; Morris *et al.*, 1977; Milas & Rinaudo, 1979; Norton *et al.*, 1984). With the polymer in this soluble ordered form, the solutions show characteristic 'weak gel' properties (Ross-Murphy *et al.*, 1983; Morris, 1991), generated by cation-mediated association of helical sequences to form a tenuous intermolecular network.

Xanthan was first launched as a commercial polysaccharide by Kelco Inc. (now The NutraSweet Kelco Company, a unit of Monsanto). In the present work, we describe the effect of side-chains and/or pendant substituents on the conformation, interactions and rheological properties of three structurally-related bacterial polysaccharides developed more recently by the same company (Pettitt, 1986). The parent member of the series is gellan gum (formerly known as S-60), the extracellular polysaccharide from the microorganism *Sphingomonas elodea* (ATCC 31461), which has previously been known as *Pseudomonas elodea* or *Auromonas elodea*. The primary structure of gellan (O'Neill *et al.*, 1983; Jansson *et al.*, 1983) has the linear tetrasaccharide repeating sequence: →3)-β-D-Glc*p*-(1→4)-β-D-Glc*p*A-(1→4)-β-D-Glc*p*-(1→4)-α-L-Rha*p*-(1→.

The polymer is biosynthesised with an L-glyceryl substituent on O(2) of the 3-linked glucose and, in at least a proportion of the repeat units, an acetyl group on O(6) of the same residue (Kuo *et al.*, 1986). In normal production of commercial gellan gum, both substituents are removed almost completely by exposure to alkali.

The other materials studied, welan and rhamsan, both have the same polysaccharide backbone as gellan, but with regularly-spaced carbohydrate side-chains. Welan (which is produced by *Alcaligenes* ATCC 31555 and was formerly known as S-130) has a single-sugar side-chain at O(3) of the 4-linked glucose (O'Neill *et al.*, 1986). The sugar may be either α-L-rhamnose or α-L-mannose, in the approximate ratio 2:1. At least 85% of the repeat units also have an acetyl substituent (Stankowski & Zeller, 1992) at O(2) of the 3-linked glucose (the same position as the glyceryl group in native gellan). In rhamsan (from *Alcaligenes* ATCC 31961 and formerly known as S-194) the side-chain is a disaccharide, β-D-Glc*p*-(1→6)-α-D-Glc*p*, which is attached at O(6) of the 3-linked glucose (the same position as the acetyl group in native gellan). The rhamsan molecule also contains acetate substituents (approximately 1 per repeat unit, on average), but these appear, from NMR evidence, to be distributed over several different sites of attachment (Jansson *et al.*, 1986).

The present paper was prepared as an invited contribution to the *International Workshop on Gellan and Related Polysaccharides*, Osaka, Japan, 14–15 November 1994, and combines an overview of previous published work from this Laboratory with a preliminary account of more recent research which will be reported in greater detail later.

MATERIALS AND METHODS

Samples of xanthan, welan and rhamsan, and of gellan with different degrees of acyl substitution, were kindly supplied by Kelco. Most experiments were carried out using the samples as received. Any modifications made for specific investigations are described later, in the appropriate sections. Solutions were prepared by mechanical stirring at ambient temperature. All reagents were AnalaR grade from BDH. Distilled deionised water was used throughout.

Differential scanning calorimetry (DSC) measurements were made using a Seteram microcalorimeter. Optical rotation was measured at 436 nm on a Perkin–Elmer 241 polarimeter, using a jacketed cell of pathlength 10 cm. Temperature was controlled by a Haake circulating water bath and measured using a thermocouple in the neck of the cell. Reading were taken after thermal equilibration at each temperature (typically 5 min).

Measurements of steady-shear viscosity were made on a Sangamo Viscoelastic Analyser, using cone-and-plate geometry of 50 mm diameter and 2 degree cone angle. Low amplitude oscillatory measurements of storage modulus (G'), loss modulus (G'') and complex dynamic viscosity (η^*) were made using cone-and-plate geometry (diameter 50 mm; cone angle 0.05 rad) on a sensitive prototype rheometer designed and constructed in this Department by Dr R.K. Richardson. To circumvent problems of thermal expansion/contraction during heating and cooling scans, the cone was truncated over 45% of its diameter, giving a gap of 0.5 mm between the flat surfaces of the two elements, but keeping strain constant at a fixed, maximum value across the outer portion (which constitutes 80% of the total area). The periphery of the sample was coated with light silicone oil to minimise loss of solvent or absorption of atmospheric moisture. Temperature was again controlled by a circulating water bath and measured by a thermocouple in direct contact with the stationary element.

EFFECT OF ACYL SUBSTITUENTS ON GELLAN

Gelation of deacylated gellan

Oriented fibres of deacylated gellan (commercial gellan gum) give high-quality, polycrystalline X-ray diffraction

patterns, which show conclusively that the polymer exists in the solid state as a co-axial double helix with 3-fold symmetry (Chandrasekaran *et al.*, 1988a, b). Under certain ionic conditions (e.g. low concentrations of Na^+ or higher concentrations of Me_4N^+ counterions), the formation and melting of this ordered structure can be observed, by techniques such as optical rotation, circular dichroism or DSC (Crescenzi *et al.*, 1987; Robinson *et al.*, 1991; Manning, 1992), as a thermally-reversible conformational transition in solution, with close superposition of the temperature-course of conformational change on heating and cooling.

The onset of gelation at higher concentrations of salt, however, is accompanied by development of thermal hysteresis between the disorder–order and order–disorder transitions and, as illustrated in Fig. 1, gel melting (and the associated loss of conformational order) occurs in two discrete steps. The first is roughly coincident with the gelation process on cooling, and can be attributed to melting of isolated sequences of double-helix structure. The second is displaced to higher temperature, and seems likely to arise from dissociation of cation-mediated helix–helix aggregates (Robinson *et al.*, 1991; Manning, 1992).

The ionic conditions required to induce aggregation and gel formation (with associated thermal hysteresis and two-stage melting) depend heavily on the nature of the cations present (Sanderson & Clark, 1984; Grasdalen & Smidsrød, 1987). Divalent metal ions such as Ca^{2+} are particularly effective, and give maximum gel strength when present at a concentration roughly equivalent to the full stoichiometric requirement of the carboxyl groups on the polymer (e.g. at ~3 mM for a typical gelling concentration of ~0.4 wt% gellan). The onset of gel formation with monovalent metal ions such as Na^+ occurs (Manning, 1992) at much higher salt concentration (~65 mM), and with Me_4N^+ as sole counterion the threshold concentration (Crescenzi *et al.*, 1987) is about an order of magnitude higher again (~600–700 mM).

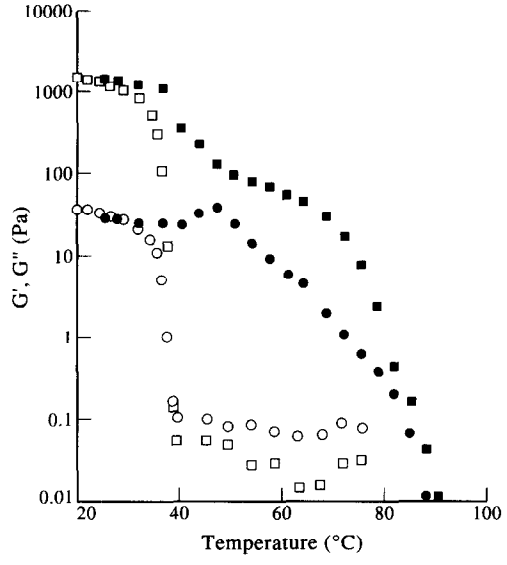

Fig. 1. Thermal hysteresis in gelation and melting of deacylated gellan (0.6 wt%), characterised by the variation in G' (squares) and G'' (circles) on cooling (open symbols) and heating (filled symbols). Measurements were made at 10 rad s^{-1} and 2% strain. The gellan sample used (batch number 66044A) had a divalent cation content equivalent to 66% of the stoichiometric requirement of the polymer carboxyl groups, and was dissolved in deionised water, with no additional salt.

These wide differences in the ability of different cations to induce gelation of deacylated gellan may be explained as follows:

(1) Divalent metal ions promote aggregation by site-binding between pairs of carboxyl groups on neighbouring helices, to give structures analogous to the 'egg-box' model proposed for calcium-induced gelation of alginate and pectin (Grant *et al.*, 1973). Computer modelling has shown that a packing arrangement of this type is sterically feasible for Ca^{2+} gellan (Chandrasekaran & Thailambal, 1990).
(2) Monovalent metal ions bind to the surface of individual helices, thus lowering their charge-density and reducing the electrostatic barrier to aggregation.
(3) Tetramethylammonium ions are incapable of forming co-ordination complexes with the carboxyl groups of gellan, but at very high concentration, can promote helix–helix aggregation by non-specific screening of electrostatic repulsion.

In all cases, however, the effect of aggregation is to stabilise the associated helices to temperatures above those at which individual helices will form on cooling (Morris & Norton, 1983), causing thermal hysteresis, and to provide an additional mechanism of interchain association, giving rise to a continuous gel network.

Gelation of 'high acyl' gellan

The acyl groups present on gellan as biosynthesised cause massive changes in the thermal stability of the double helix and in its ability to form cation-mediated aggregates. The sample used in the present work was exposed to high temperature during extraction from the culture broth, and will therefore be referred to as 'high acyl' rather than 'native'.

As shown in Fig. 2, the disorder–order and order–disorder transitions accompanying gelation and melting of this material follow the same temperature course, with no detectable thermal hysteresis, and addition of high concentrations of salt causes only a slight increase in transition temperature. Conformational ordering, however, occurs at much higher temperature than for commercial gellan gum (Fig. 3). It would therefore appear that the double helix structure of high acyl gellan is inherently more stable than that of the deacylated material, but that it has little, if any, capacity for cation-mediated aggregation. Consistent with this inter-

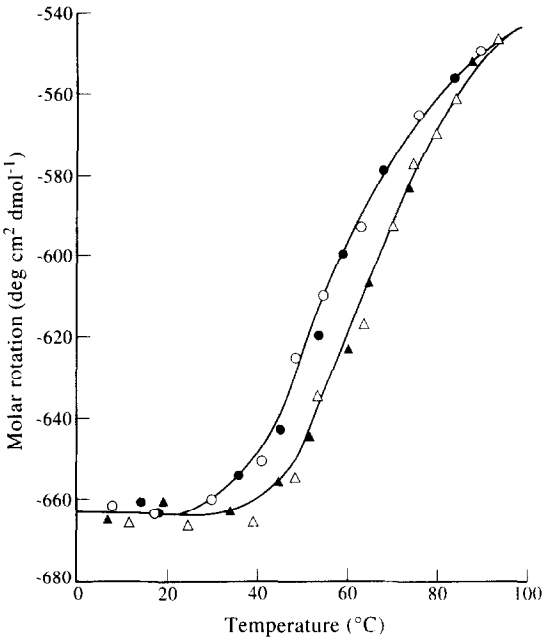

Fig. 2. Variation of optical rotation (436 nm) with temperature for high acyl gellan (1.0 wt%) on heating (filled symbols) and cooling (open symbols) in deionised water (circles) and in 100 mM NaCl (triangles).

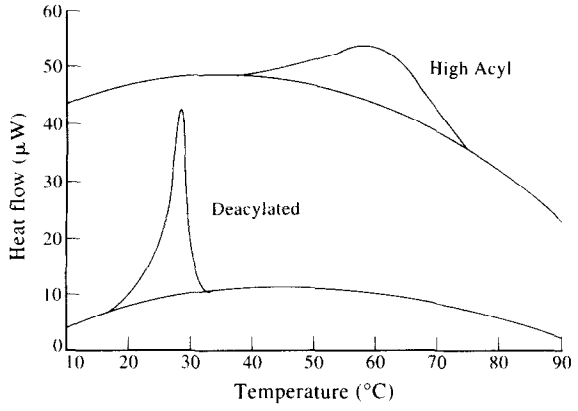

Fig. 3. DSC scans for high acyl and deacylated gellan (1.0 wt% in deionised water) on cooling at 0.1°C/min. Both samples were in the Na^+-salt form (prepared by cation exchange on Amberlite IR 120 resin).

pretation, the gels formed by high acyl gellan are far weaker and less rigid than those of commercial gellan gum, and the polymer concentrations required for gelation are substantially higher (Baird *et al.*, 1992).

Mixtures of high acyl and deacylated gellan show two separate conformational transitions at temperatures characteristic of the individual components (Fig. 4), with no indication of the formation of double helices involving strands of both types.

The helical structure of high acyl gellan was first explored by using computer modelling to extrapolate from the known solid-state geometry of the deacylated polymer (Chandrasekaran & Thailambal, 1990). The main conclusion was that acetate groups would lie on the periphery of the duplex (Fig. 5), with no modification of the underlying helix geometry, but that accommodation of glycerate would require the carboxyl group on the adjacent glucuronate residue to swing round through an angle of ~30° about the C(5)–C(6) bond, with consequent major changes in the pattern of hydrogen-bonding within and between the participating strands. When diffraction patterns of the quality needed for detailed analysis were subsequently obtained (Chandrasekaran *et al.*, 1992), it was found that the actual rotation of the carboxyl group is less than predicted (~14°), but that the required separation from the glycerate substituent is achieved by the glucuronate residue itself also undergoing a significant rotation (17±3°) about the glycosidic bonds linking it to the two adjacent glucose residues. This modification of helix geometry would explain the difference in thermal stability of the ordered structures (Fig. 3) and the absence of

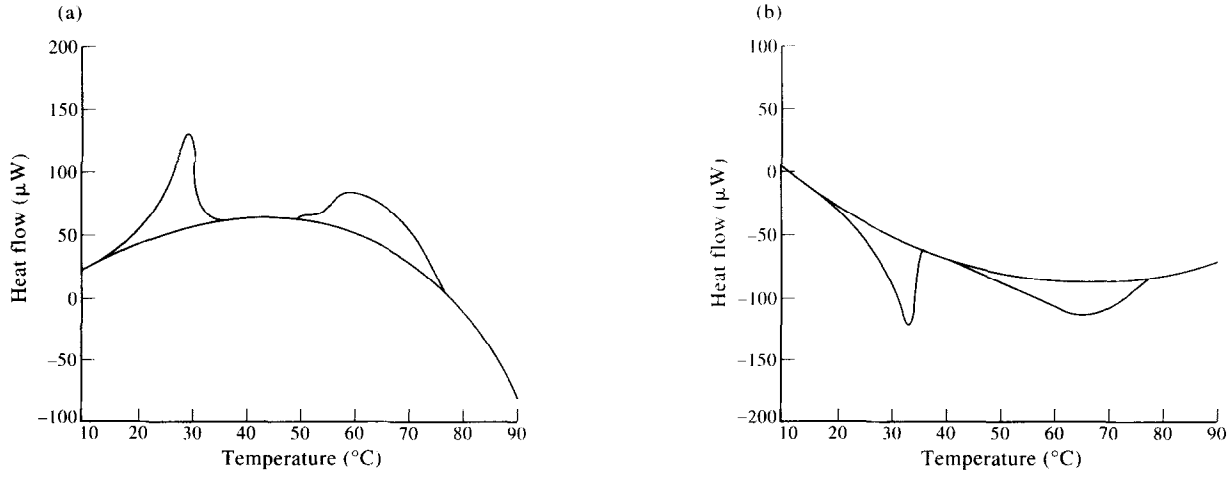

Fig. 4. DSC traces for a mixture of high acyl and deacylated gellan on (a) cooling and (b) heating at 0.3°C/min. Both samples were in the Na^+ salt form and were present at a concentration of 1.0 wt% in deionised water.

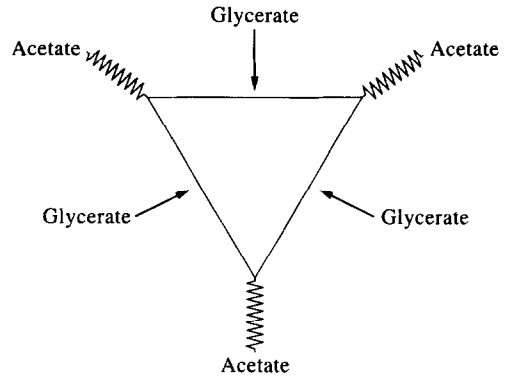

Fig. 5. Schematic representation of the positions of acetate and glycerate substituents on the double-helix structure of high acyl gellan, as viewed along the axis of the 3-fold helix.

interaction between acylated and deacylated chains (Fig. 4).

Partially-deacylated gellan samples

Suppression of helix–helix aggregation in high acyl gellan could, in principle, arise in two different ways. The most obvious interpretation is that the acetyl groups on the surface of the helix promote solvation and block close-packing, as described previously for chemical substituents in cellulose derivatives. An alternative possibility, however, is that glyceryl substituents alter the stereochemical structure in the vicinity of the carboxyl groups to a form that is incapable of accommodating site-bound counterions. To distinguish between these two possibilities we have examined the behaviour of gellan samples prepared under conditions that promote selective removal of either acetate or glycerate.

In normal commercial production of gellan gum, acyl groups are removed by brief exposure to alkali (KOH) at high temperature (~95°C). The reaction can be expressed as:

$$\text{R-COO-R}' + \text{KOH} \rightarrow \text{R-COOK} + \text{HOR}'$$

where R and R′ denote, respectively, the substituent and the polymer chain. Partial removal can be achieved by limiting the amount of alkali used. When hydrolysis is carried out in this way, at elevated temperature with the polymer in the disordered form, glyceryl substituents are liberated somewhat more rapidly than acetyl groups. An alternative procedure, however, is to hydrolyse for much longer times at lower temperature, where the polymer is conformationally ordered. Under these conditions, release of acetate groups from the periphery of the helical duplex occurs far more rapidly than removal of glyceryl substituents embedded within the helix. Thus by appropriate manipulation of temperature, alkali concentration, and hydrolysis time (Baird *et al.*, 1992), it is possible to obtain samples differing widely in the proportion of repeat units carrying residual acetate or glycerate substituents (denoted here as, respectively, f_a and f_g).

Figure 6 shows the temperature-course of gelation and melting for two preparations with, respectively, $f_g = 0.43$, $f_a = 0.03$, and $f_g = 0.52$, $f_a = 0.50$. Although both have a similar, substantial, content of residual glycerate, the first sample, which is virtually devoid of acetate, shows pronounced thermal hysteresis (Fig. 6a), but no hysteresis is detectable (Fig. 6b) for the second sample, which has a high content of acetate. It would therefore appear that the presence of acetyl groups on the periphery of the double helix is the dominant factor in blocking helix–helix aggregation. However, the temperature-course of gelation is closely similar for both samples, indicating that acetate groups have little influence on the formation of individual double helices, and that the enhanced stability of the helix structure in high acyl gellan is due predominantly, or solely, to glycerate.

The effect of glyceryl substituents on the thermal stability of the gellan double helix was explored in

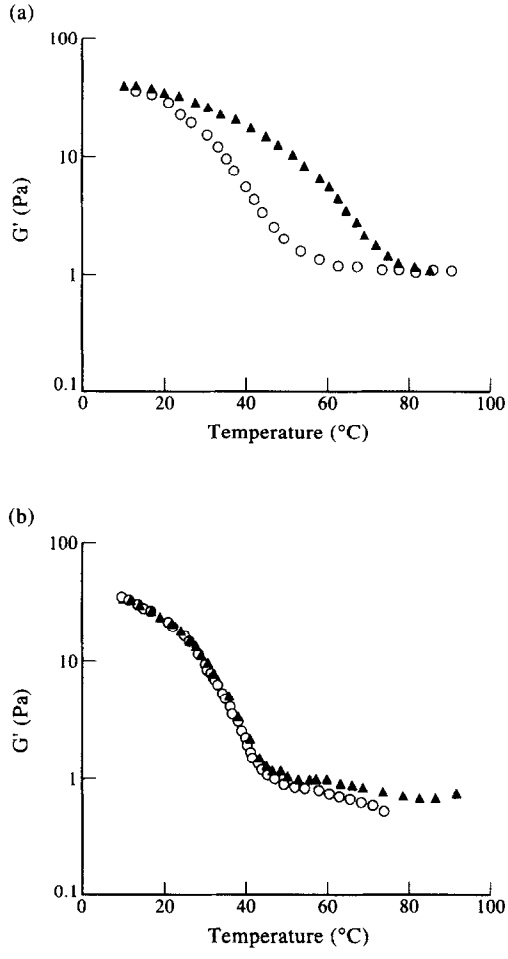

Fig. 6. Variation of G' (10 rad s^{-1}; 2% strain) on cooling (○) and heating (▲) at 1°C/min for partially deacylated gellan preparations (0.5 wt% in deionised water) with acyl contents (% stoichiometric) of (a) 40% glycerate, 3% acetate and (b) 52% glycerate, 50% acetate.

Table 1. Samples with progressive removal of glycerate

Sample Kelco batch number	A 89–1444	B 89–1445	C 89–1446	D 89–1447	E 89–1448
Hydrolysis time (h) (25 mM KOH; 40°C)	4	10	24	48	72
Acyl content (% stoichiometric)					
Glycerate	61.5	43.4	19.4	8.3	3.8
Acetate	< 1	< 1	< 1	< 1	< 1

greater detail by using DSC to monitor conformational ordering of a series of gellan samples prepared from the same fermentation broth by progressively longer exposure to alkali (25 mM KOH) at 40°C, where the polymer is in the ordered form. The shortest hydrolysis time used (4 h) was sufficient to give almost complete removal of acetyl groups from the periphery of the helix. The glycerate level, however, decreased systematically (Table 1) from over 60% of full stoichiometric equivalence in the first sample (A) to below 4% in the final sample (E).

Figure 7 shows the DSC cooling scans recorded for these materials. At glycerate levels above ~40% stoichiometric (samples A and B) the traces show a large exotherm at high temperature, widely separated from a much smaller peak centred close to 20°C. On further reduction of glycerate content to below ~20% stoichiometric (samples C and D) the two peaks overlap, but with obvious increase in the relative contribution of the second transition to overall thermal change. Finally, at low glycerate content (< 4% stoichiometric; sample E) the peaks merge, but the position and width of the exotherm suggest that it is still a composite of two (heavily overlapping) transitions.

As shown in Fig. 7, the first, major transition moves to progressively lower temperature with decreasing content of glycerate, whereas the temperature of the second, smaller transition remains essentially constant. The obvious interpretation is that the first process corresponds to formation of the 'high acyl' structure, with progressive reduction in stability as the proportion of missing glycerate groups increases, and that the second comes from ordering of chain sequences totally devoid of glycerate.

On the assumption that this proposal is correct, we can obtain a rough estimate of the minimum sequence length required for adoption of the fully-deacylated helix structure. If the fraction of tetrasaccharide units lacking glycerate substituents ($1-f_g$) is denoted as f, then the probability of finding n such units adjacent to one another in the polymer chain is f^n. This can be compared with the relative contribution of the second transition to overall thermal change to give an approximate value of n.

The two DSC peaks for sample C (Fig. 7) are particularly well resolved, and can be quantified with good precision. The lower-temperature transition contributes ~26% of the total heat change (i.e. $f^n \approx 0.26$). The glycerate content of this sample (Table 1) is ~19.4% stoichiometric (i.e. $f = 0.806$), yielding excellent agreement ($f^n = 0.27$) with the 'target' value if the exponent is set at $n = 6$. A consecutive run of 6 glycerate-free repeating units on each of the participating strands would correspond to two full turns of the deacylated double-helix structure, which seems a physically-realistic length for stable association.

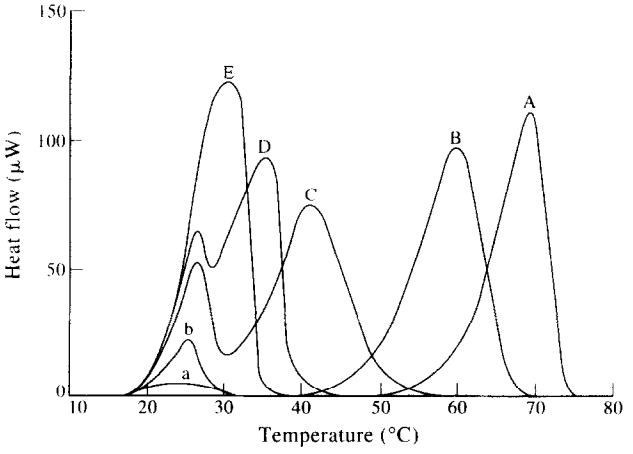

Fig. 7. DSC cooling scans (0.7°C/min) for gellan samples devoid of acetate substituents, but with progressive reduction in glycerate content (Table 1) from ~61.5% stoichiometric in A to ~3.8% in E. The minor exotherms observed at low temperature for samples A and B are identified by the corresponding lower-case letters.

In summary, it would appear that gellan can adopt two different, mutually incompatible helical structures, the 'high acyl' form which is stabilised by participation of glycerate groups in interchain association, and the deacylated form which cannot accommodate glycerate substituents. Sequences of 6 or more repeat units devoid of glycerate terminate the 'high acyl' structure, but are sufficient for stable association in the deacylated arrangement. Shorter runs of glycerate-free units can be accommodated in the 'high acyl' structure, but reduce its stability. Acetyl substituents remain conformationally mobile on the periphery of the duplex, and block helix–helix aggregation.

CONFORMATION AND RHEOLOGY OF WELAN AND RHAMSAN

Rheological properties in aqueous solution

In contrast to the behaviour outlined above for the unbranched parent polysaccharide, aqueous solutions of the branched gellan variants, welan and rhamsan, show no evidence of gel formation or conformational change on heating and cooling. One obvious interpretation (Crescenzi *et al.*, 1987) would be that both polymers exist in solution as disordered coils. There is now overwhelming evidence, however, that incorporation of side-chains in the ordered structure stabilises the double helical conformation of the polymer backbone to temperatures above 100°C.

First, the solution properties of welan and rhamsan are strikingly similar to those of the 'weak gel' networks formed by ordered xanthan (Ross-Murphy *et al.*, 1983). As shown in Fig. 8, both give gel-like mechanical spectra, with $G' > G''$ and little frequency-dependence in either modulus, in contrast to the strong frequency-dependence typical of disordered coils. Also, the values of complex dynamic viscosity (η^*), obtained from low-amplitude oscillatory measurements which conserve intermolecular association, are substantially higher than the corresponding values of steady-shear viscosity (η) from rotational measurements where network structure is disrupted. This is again in direct contrast to the behaviour of disordered coils interacting solely by topological entanglement, where $\eta \approx \eta^*$ at equivalent numerical values of shear-rate ($\dot{\gamma}/s^{-1}$) and frequency (ω/rad s^{-1}), a generality known as the Cox–Merz rule (Cox & Merz, 1958).

The increase in G' (characterising solid-like response) with increasing concentration (c) is also closely similar for welan, rhamsan and ordered xanthan. At concentrations within the range 0.2–2.0 wt%, all three polymers give linear plots of log G' versus log c (Fig. 9) and in each case the slope is ~2.0 (i.e. $G' \propto c^2$), as commonly found for normal polysaccharide gels at concentrations well above the minimum critical gelling concentration, c_0 (Clark & Ross-Murphy, 1987).

As might be anticipated from the absence of any detectable conformational change on heating and cooling, the 'weak gel' properties of welan and rhamsan show little variation with temperature (Fig. 10). They are also virtually independent of ionic environment, as illustrated in Fig. 11 for welan in the presence of NaCl at concentrations ranging from 0 to 1 M.

Conformational transitions and gel formation

We have found recently that the ordered structure of welan can be disrupted by dissolving the polymer in dimethyl sulphoxide (DMSO), rather than in water (Hember *et al.*, 1994; Hember & Morris, 1995). As shown in Fig. 12, the solution properties are then typical of a disordered polysaccharide; G'' is substantially higher than G', both moduli increase steeply with increasing frequency, and there is close Cox–Merz superposition of $\eta(\dot{\gamma})$ and $\eta^*(\omega)$, both of which level out towards a constant Newtonian value at low rates.

In mixed solvents of water and DMSO, loss of 'weak gel' structure (characterised by large reductions in G' and G'' and a large increase in tan δ, the ratio of G''/G') occurs abruptly over a narrow range of composition (Fig. 13). Solutions of welan prepared at a DMSO/water ratio just below this critical range show typical order–disorder and disorder–order transitions on heating and cooling (Fig. 14). There is substantial thermal hysteresis between formation and melting of the ordered structure, indicative of helix–helix aggregation as in

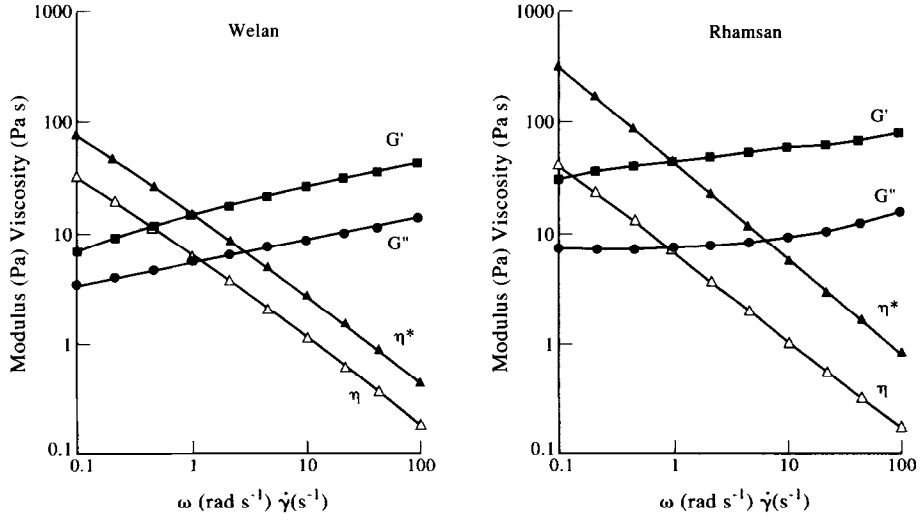

Fig. 8. Mechanical spectra (25°C; 2% strain) for welan and rhamsan (1.0 wt% in deionised water), showing the variation of G' (■), G'' (●) and η^* (▲) with frequency (ω). The variation of steady-shear viscosity (η) with shear rate ($\dot{\gamma}$) is also shown (△).

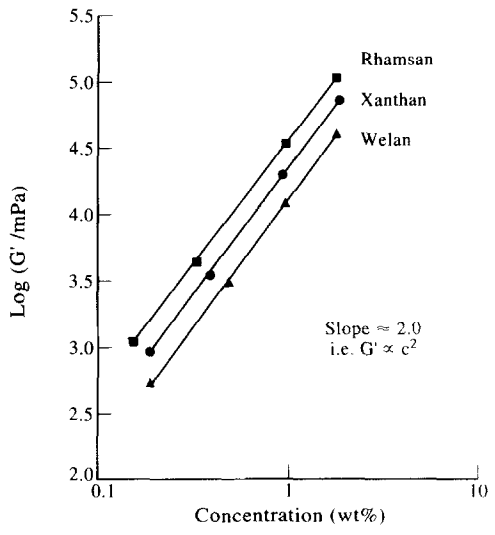

Fig. 9. Concentration-dependence of G' (1.0 rad s^{-1}; 2% strain; 25°C) for xanthan (●), welan (△), and rhamsan (■).

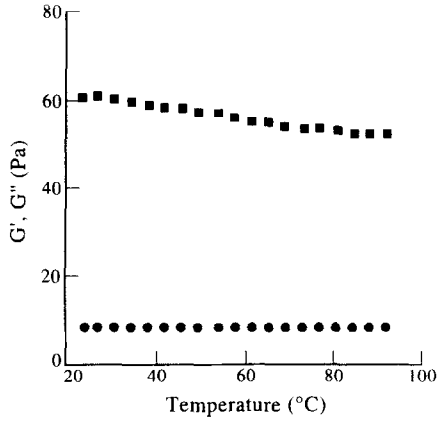

Fig. 10. Temperature-dependence of G' (■) and G'' (●) for rhamsan (1.0 wt% in deionised water). Measurements were made at 1 rad s^{-1} and 2% strain. Similar featureless traces were obtained for welan.

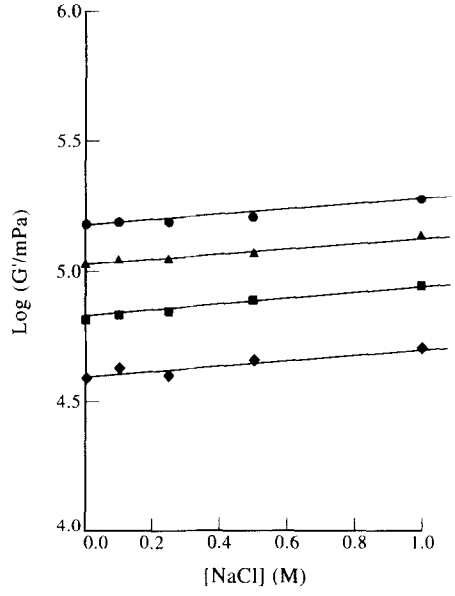

Fig. 11. Effect of salt concentration on the 'weak gel' properties of welan (2.0 wt%; 25°C) in water, as monitored by measurements of G' (2% strain) at frequencies (rad s^{-1}) of 0.1 (◆), 1.0 (■), 10 (▲) and 100 (●). The rheology of rhamsan also showed little variation with ionic environment. From Hember & Morris (1995), with permission.

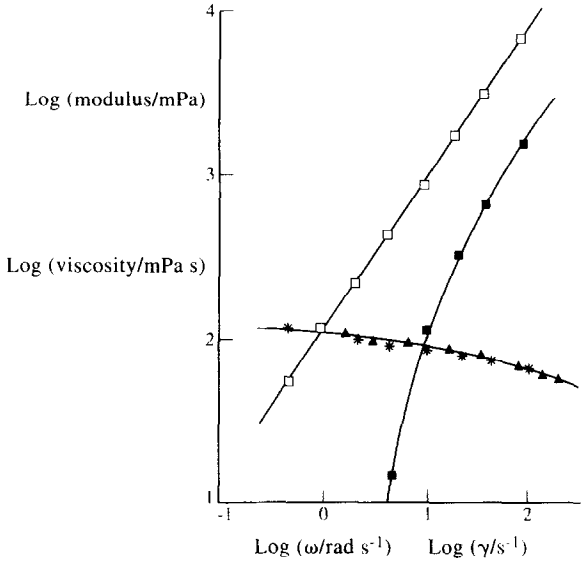

Fig. 12. Solution rheology of welan (1.0 wt%; 25°C) in DMSO, showing the variation of G' (■), G'' (□) and η^* (✱) with frequency (ω), and of η (▲) with shear rate ($\dot{\gamma}$). From Hember *et al.* (1994), with permission.

deacylated gellan (Fig. 1). This may be due, at least in part, to the increased strength of electrostatic attraction between the polyanion and its surrounding counterions as the dielectric constant of the solvent is decreased by replacing water with DMSO (Hember & Morris, 1995).

Observation of a co-operative conformational transition in solution is, of course, one of the most direct and convincing ways of establishing the existence of an ordered polysaccharide structure under hydrated conditions. We therefore carried out analogous experiments on rhamsan, but found that, although the polymer would dissolve in DMSO, the solutions retained the 'weak gel' properties indicative of conformational rigidity.

The ordered structure of rhamsan can, however, be destabilised by removal of the acetate substituents, and the polymer then shows sigmoidal changes in optical rotation on heating and cooling (Campana *et al.*, 1992). In the present work, we have explored the effect of the order–disorder and disorder–order processes on the rheological properties of rhamsan, using a sample prepared by alkaline hydrolysis in aqueous solution (0.1 M KOH; 4 days; 25°C). These conditions are less severe than the treatment used by Campana *et al.* (1992) (hydrolysis in 5:1 DMSO:water), but our main concern was to avoid the possibility of side-reactions and maintain the integrity of the ordered structure at

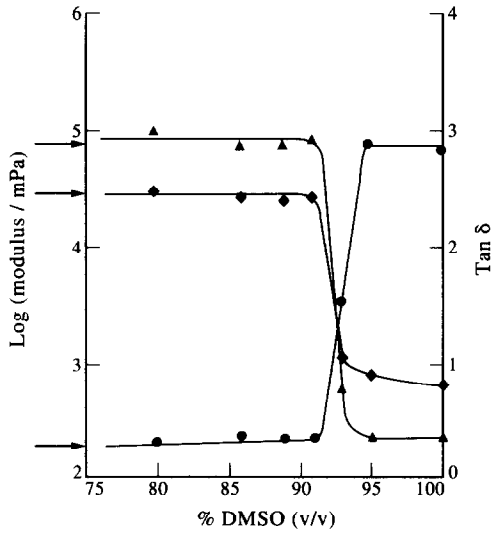

Fig. 13. Variation of G' (▲), G'' (◆), and tan δ (●) with solvent composition for welan (2.0 wt%; 25°C) in mixtures of DMSO and water. Measurements were made at 1 rad s^{-1} and 2% strain. The arrows at the left-hand axis show the values obtained in pure water. From Hember & Morris (1995), with permission.

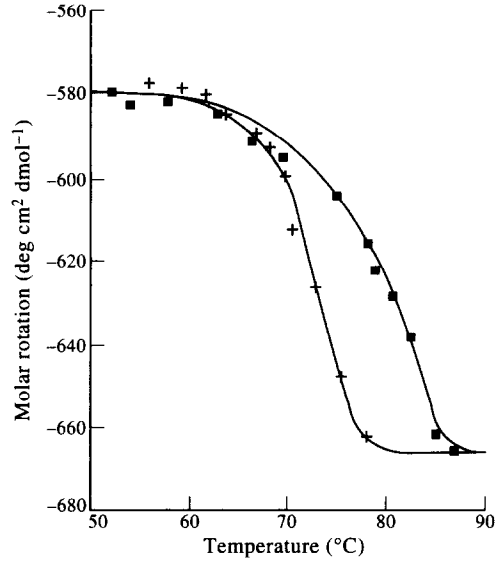

Fig. 14. Temperature-dependence of optical rotation (436 nm) during heating (■) and cooling (+) for welan (0.5 wt%) in 6:1 DMSO:water. From Hember *et al.* (1994), with permission.

the temperature of the reaction, rather than to achieve complete deacetylation.

The mechanical spectrum of the resulting material (1.0 wt% in water at 25°C) displayed gel-like character similar to that shown in Fig. 8b for the unmodified polymer, indicating that the ordered structure had, indeed, remained intact during the hydrolysis procedure. DSC traces (Fig. 15) show a well-defined endotherm on heating, with a corresponding exotherm on cooling, confirming that the extent of deacetylation achieved was sufficient to bring the order–disorder and

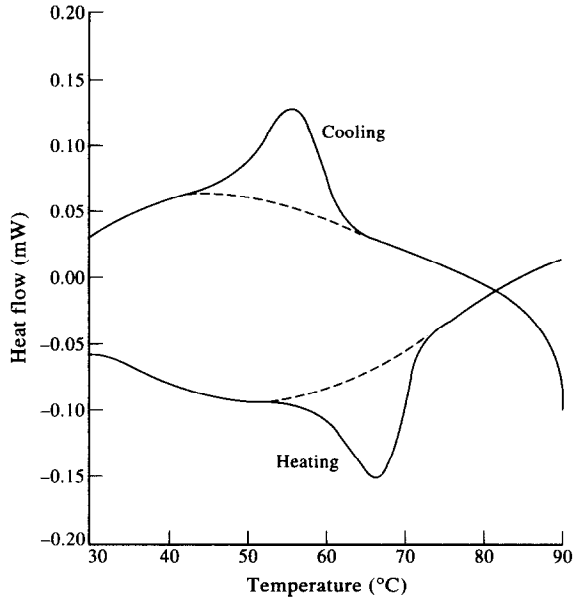

Fig. 15. DSC traces for deacetylated rhamsan (1.0 wt% in deionised water) on heating and cooling at 0.5°C/min.

disorder–order transitions into an accessible temperature range. As found for welan in aqueous DMSO (Fig. 14), the melting process on heating occurs at significantly higher temperature than the ordering process on cooling, again indicating helix–helix aggregation.

The heating endotherm in DSC is accompanied (Fig. 16) by a massive reduction in G' and G'', corresponding to loss of the 'weak gel' network. The moduli increase again on cooling, over approximately the same temperature range as the exotherm in DSC. The resulting network, however, is a 'true', self-supporting gel, rather than the pourable 'weak gel' structure present initially. Although the values of G' are similar before and after heating, the difference in network properties is reflected in the lower final value of G'' (i.e. lower tan δ), and is obvious by simple visual inspection.

Interpretation and conclusions

X-ray fibre diffraction studies (Chandrasekaran *et al.*, 1994) have shown that, in the solid state, welan adopts an ordered conformation closely similar to that of gellan, but with additional stabilisation of the double helix by non-covalent interactions with the carbohydrate side-chains. The investigations described in the present work demonstrate that the ordered structure remains intact in aqueous solution, but can be dissociated by DMSO. Current diffraction patterns for rhamsan (Lee & Chandrasekaran, 1991) are less well resolved than those of gellan and welan, but show the same general features, indicating similar double-helical geometry. Our present results again indicate that the ordered structure persists under hydrated conditions.

We have observed previously (Hember *et al.*, 1994)

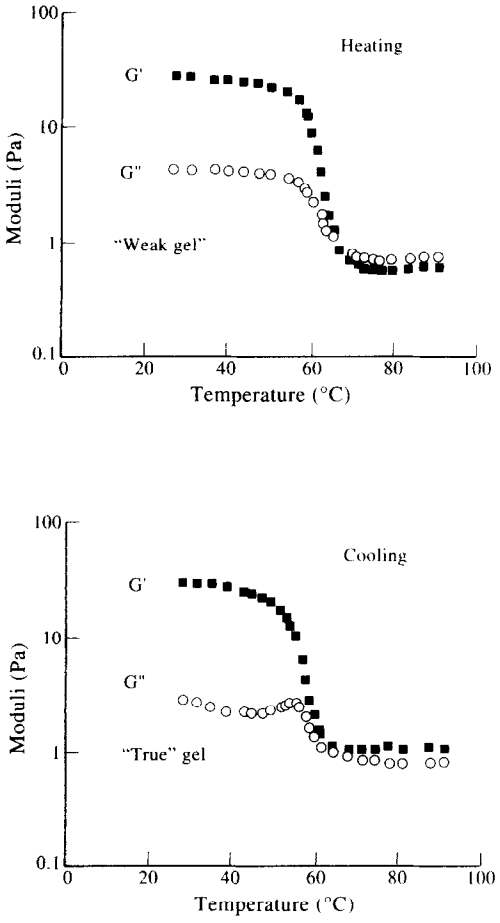

Fig. 16. Variation of G' (■) and G'' (○) for deacetylated rhamsan (0.5 wt% in deionised water) on initial heating and subsequent cooling (1°C/min).

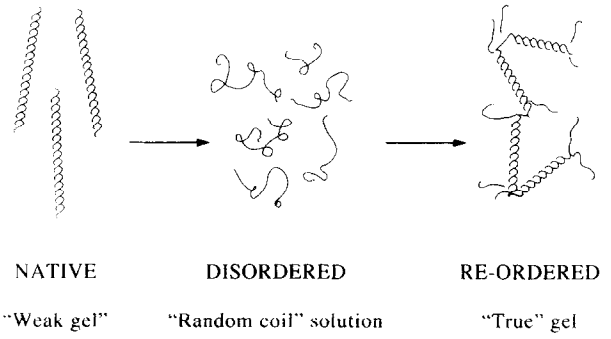

Fig. 17. Schematic representation of proposed mechanism for denaturation and gelation of welan and rhamsan. From Hember *et al.* (1994), with permission.

that if solutions of disordered welan in DMSO are poured into water (causing rapid regeneration of double-helix structure), they give cohesive strands of gel, which remain intact on prolonged storage in water. However, as described above, helix–helix interactions in aqueous solutions of ordered welan prior to denaturation are confined to development of a 'weak-gel' network. Our interpretation of this behaviour is that the native structure of welan is a perfect double helix, with exact pairing of chains along their entire length; dissociation and regeneration of double-helix geometry allows development of a crosslinked network by individual chains forming shorter helices with more than one partner (Fig. 17). The conversion of deacetylated rhamsan from a 'weak gel' to a 'true' gel after heating and cooling through the temperature range of the order–disorder transition (Figs 15 and 16) can be explained in the same way, again arguing for exact pairing of strands in the ordered structure of the native polymer.

Finally, the reduction in thermal stability of the rhamsan double-helix by deacetylation suggests that, like the glycerate groups in native gellan, the acetate groups in rhamsan form an integral part of the ordered structure. Detailed interpretation of the way in which these substituents interact with the polymer backbone will, however, remain impossible until their sites of attachment are determined. This is an obvious target for future research.

ACKNOWLEDGEMENTS

We thank Kelco for provision of samples, financial support of this investigation, and many stimulating discussions. We also thank our colleague Dr R.K. Richardson for generous access to his prototype rheometer.

REFERENCES

Baird, J.K., Talashek, T.A. & Chang, H., (1992). Gellan gum: effect of composition on gel properties. In *Gums and Stabilisers for the Food Industry 6*, eds G.O. Phillips, P.A. Williams & D.J. Wedlock. IRL Press, Oxford, pp. 479–487.

Campana, S., Ganter, J., Milas, M. & Rinaudo, M. (1992). On the solution properties of bacterial polysaccharides of the gellan family. *Carbohydr. Res.*, **231**, 31–38.

Chandrasekaran, R., Millane, R.P., Arnott, S. & Atkins, E.D.T. (1988a). The crystal structure of gellan. *Carbohydr. Res.*, **175**, 19881–15.

Chandrasekaran, R., Puigjaner, L.C., Joyce, K.L. & Arnott, S. (1988b). Cation interactions in gellan: an X-ray study of the potassium salt. *Carbohydr. Res.*, **181**, 23–40.

Chandrasekaran, R., Radha, A. & Lee, E.J. (1994). Structural roles of calcium ions and side chains in welan: an X-ray study. *Carbohydr. Res.*, **252**, 183–207.

Chandrasekaran, R., Radha, A. & Thailambal, V.G. (1992). Roles of potassium ions, acetyl and L-glyceryl groups in native gellan double helix: an X-ray study. *Carbohydr. Res.*, **224**, 1–17.

Chandrasekaran, R. & Thailambal, V.G. (1990). The influence of calcium ions, acetate and L-glycerate groups on the gellan double-helix. *Carbohydr. Polym.*, **12**, 431–442.

Clark, A.H. & Ross-Murphy, S.B. (1987). Structural and mechanical properties of biopolymer gels. *Advan. Polym. Sci.*, **83**, 57–192.

Cox, W.P. & Merz, E.H. (1958). Correlation of dynamic and steady-flow viscosities. *J. Polym. Sci.*, **28,** 619–622.

Crescenzi, V., Dentini, M. & Dea, I.C.M. (1987). The influence of side-chains on the dilute-solution properties of three structurally related, bacterial anionic polysaccharides. *Carbohydr. Res.*, **160,** 283–302.

Dea, I.C.M. & Morrison, A. (1975). Chemistry and interactions of seed galactomannans. *Adv. Carbohydr. Chem. Biochem.*, **31,** 241–312.

Grant, G.T., Morris, E.R., Rees, D.A., Smith, P.J.C. & Thom, D. (1973). Biological interactions between polysaccharides and divalent cations: the egg-box model. *FEBS Lett.*, **32,** 195–198.

Grasdalen, H., Smidsrød, O. (1987). Gelation of gellan gum. *Carbohydr. Polym.*, **7,** 371–393.

Hember, M.W.N. & Morris, E.R. (1995). Solubility, solution rheology and salt-induced gelation of welan polysaccharide in organic solvents. *Carbohydr. Polym.*, **27,** 23–36.

Hember, M.W.N., Richardson, R.K. & Morris, E.R. (1994). Native ordered structure of welan polysaccharide: conformational transitions and gel formation in aqueous dimethyl sulphoxide. *Carbohydr. Res.*, **252,** 209–221.

Holzwarth, G. (1976). Conformation of the extracellular polysaccharaide of *Xanthomonas campestris. Biochemistry,* **15,** 4333–4339.

Jansson, P.-E., Kenne, L. & Lindberg, B. (1975). Structure of the extracellular polysaccharide from *Xanthomonas campestris. Carbohydr. Res.*, **45,** 275–282.

Jansson, P.-E., Lindberg, B., Lindberg, J., Maekawa, E. & Sandford, P.A. (1986). Structural studies of a polysaccharide (S-194) elaborated by *Alcaligenes* ATCC 31961. *Carbohydr. Res.*, **156,** 157–163.

Jansson, P.-E., Lindberg, B. & Sandford, P.A. (1983). Structural studies of gellan gum, an extracellular polysaccharide elaborated by *Pseudomonas elodea. Carbohydr. Res.*, **124,** 135–139.

Jeanes, A., Pittsley, J.E. & Senti, F.R. (1961). Polysaccharide B-1459: a new hydrocolloid polyelectrolyte produced from glucose by bacterial fermentation. *J. Appl. Polym. Sci.*, **5,** 519–526.

Kuo, M.-S., Mort, A.J. & Dell, A. (1986).Identification and location of L-glycerate, an unusual acyl substituent in gellan gum. *Carbohydr. Res.*, **156,** 173–187.

Lee, E.J. & Chandrasekaran, R. (1991). X-ray and computer modelling studies on gellan-related polymers: molecular structures of welan, S-657, and rhamsan. *Carbohydr. Res.*, **214,** 11–24.

Manning, C.E. (1992). *Formation and Melting of Gellan Polysaccharide Gels*, PhD Thesis, Cranfield Institute of Technology, Silsoe College, Bedford, UK.

Melton, L.D., Mindt, L., Rees, D.A. & Sanderson, G.R. (1976). Covalent structure of the extracellular polysaccharide from *Xanthomonas campestris. Carbohydr. Res.*, **46,** 245–257.

Milas, M. & Rinaudo, M. (1979). Conformational investigation on the bacterial polysaccharide xanthan. *Carbohydr. Res.*, **76,** 189–196.

Moorhouse, R., Walkinshaw, M.D. & Arnott, S. (1977). Xanthan gum—molecular conformation and interactions. *Am. Chem. Soc. Symp. Ser.*, **45,** 90–100.

Morris, E.R. (1973).Polysaccharide conformation as a basis of food structure. In *Molecular Structure and Function of Food Carbohydrate*, eds G.G. Birch & L.F. Green. Applied Science, London, pp. 125–132.

Morris, E.R. (1991). Pourable gels: polysaccharides that stabilise emulsions and dispersions by physical trapping. *Int. Food Ingredients,* **1,** 32–37.

Morris, E.R. & Norton, I.T. (1983). Polysaccharide aggregation in solutions and gels. In *Aggregation Processes in Solution*, eds E. Wyn-Jones & J. Gormally. Elsevier, Amsterdam, pp. 549–593.

Morris, E.R., Rees, D.A., Young, G., Walkinshaw, M.D. & Darke, A. (1977). Order–disorder transition for a bacterial polysaccharide in solution. A role for polysaccharide conformation in recognition between *Xanthomonas* pathogen and its hosts. *J. Mol. Biol.*, **110,** 1–16.

Nishinari, K., Williams, P.A. & Phillips, G.O. (1992). Review of the physico-chemical characteristics and properties of konjac mannan. *Food Hydrocoll.*, **6,** 199–222.

Norton, I.T., Goodall, D.M., Frangou, S.A., Morris, E.R. & Rees, D.A. (1984). Mechanism and dynamics of conformational ordering in xanthan polysaccharides. *J. Mol. Biol.*, **175,** 371–394.

O'Neill, M.A., Selvendran, R.R. & Morris, V.J. (1983). Structure of the acidic extracellular gelling polysaccharide produced by *Pseudomonas elodea. Carbohydr. Res.*, **124,** 123–133.

O'Neill, M.A., Selvendran, R.R., Morris, V.J. & Eagles, J. (1986).Structure of the extracellular polysaccharide produced by the bacterium *Alcaligenes* (ATCC 31555) species. *Carbohydr. Res.*, **145,** 295–313.

Pettitt, D.J. (1986). Recent developments—future trends. In *Gums and Stabilisers for the Food Industry 3*, eds G.O. Phillips, D.J. Wedlock & P.A. Williams. Elsevier, London, pp. 451–463.

Robinson, G., Manning, C.E. & Morris, E.R. (1991). Conformation and physical properties of the bacterial polysaccharides gellan, welan and rhamsan. In *Food Polymers, Gels and Colloids*, ed. E. Dickinson. Special Publication No. 82, Royal Society of Chemistry, Cambridge, pp. 22–33.

Ross-Murphy, S.B., Morris, V.J. & Morris, E.R. (1983). Molecular viscosity of xanthan polysaccharide. *Faraday Symp. Chem. Soc.*, **18,** 115–129.

Sanderson, G.R. & Clark, R.C. (1984). Gellan gum, a new gelling polysaccharide. In *Gums and Stabilisers for the Food Industry 2*, eds G.O. Phillips, D.J. Wedlock, & P.A. Williams. Pergamon Press, Oxford, pp. 201–210.

Stankowski, J.D. & Zeller, S.G. (1992). Location of the *O*-acetyl group in welan by reductive cleavage method. *Carbohydr. Res.*, **224,** 337–341.

Whistler, R.L. & BeMiller, J.N. (1993). *Industrial Gums: Polysaccharides and their Derivatives, 3rd Edition.* Academic Press, San Diego.