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ASSOCIATIONS OF LIKE AND UNLIKE POLYSACCHARIDES: MECHANISM AND SPECIFICITY IN GALACTOMANNANS, INTERACTING BACTERIAL POLYSACCHARIDES, AND RELATED SYSTEMS*

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ABSTRACT

Chiroptical, rheological, and n.m.r.-relaxation evidence is presented, to identify interactions of two types between different polysaccharides: (*1*) mutual exclusion of incompatible molecules, with consequent increase in the effective concentration of both; and (*2*) energetically favourable association of structurally and sterically regular chain-segments. β-1,4-linked plant polysaccharides interact by association of unsubstituted backbone regions, either with like chains, or with sterically compatible, unlike molecules. Extracellular polysaccharides (xanthans) of *Xanthomonas* plant pathogens maintain their ordered native conformation in solution, and this accounts for their industrially valuable, rheological peculiarities. These materials bind strongly to the plant glycans. Random-coil bacterial gums show no such interactions, although dextran enhances autogelation of galactomannans by exclusion. Extracellular polysaccharides from *Arthrobacter* species also have ordered native conformations in solution, but do not share the specific interactions of xanthan. Native xanthan shows marked specificity in its interactions with plant glycans, indicating a possible biological role in host–pathogen recognition.

INTRODUCTION

Despite widespread technological use of synergistic interactions between different hydrocolloids in solution, the molecular origin of such effects has, in general, remained obscure[1]. We have demonstrated, however, that interaction of plant galactomannans with agarose or κ-carrageenan to form mixed gels, at total polymer levels far below those required for gelation of either polysaccharide alone, involves

*Dedicated to the memory of Sir Edmund Hirst, C.B.E., F.R.S.
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intimate association of specific sequences of the galactomannan with native double-helical conformations of the algal polysaccharide chains[2]. Recently, we suggested a similar mechanism for the interaction of galactomannans with xanthan gum, a heteropolysaccharide of bacterial origin, and proposed a biological role for such association in substrate recognition by the synthesising bacterium, *Xanthomonas campestris*[3,4]. In order to explore the specificity or generality of this type of interaction, we have now studied a wider range of β-1,4-linked plant polysaccharides, and a wider range of bacterial polysaccharides, including some for which interactions with galactomannans have been reported[5,6]. To place any such interactions in a realistic context, we have also studied the association of like chains. For plant polysaccharides, these associations may reflect, *in vitro*, the molecular organisation *in vivo*, while the industrially valuable, rheological peculiarities of certain bacterial polysaccharides may have their origin in tenuous, but specific, intermolecular alignment and interaction in solution[3].

Interchain associations may be characterised quantitatively by a variety of rheological techniques, the appropriate technique being largely determined by the nature of the association. Thus, firm gels are conveniently characterised by their rigidity modulus or yield stress. For gelling systems that are thermally reversible, the melting point and setting temperature of the gel provide a sensitive index of intermolecular interactions. Structure in weak gels or viscoelastic fluids may be monitored by oscillatory measurements of dynamic viscosity and rigidity, while rotational measurements may be used to follow the breakdown of structure at different rates of shear.

To monitor and characterise molecular conformation, we have used chiroptical and n.m.r. techniques that have proved very successful in previous studies of polysaccharide systems. Changes in polysaccharide conformation are frequently accompanied by large changes in optical activity; in particular, single-wavelength optical rotation provides a sensitive and convenient index of chain conformation[2,7–9]. A simple, quantitative relationship has been developed to predict changes in optical rotation arising from changes in the dihedral angles between adjacent sugar residues in the polymer chain[10,11]. Circular dichroism (c.d.) measurements may be used to monitor directly changes in the geometric environment of specific groups which absorb light in an accessible, spectral region[12]. The c.d. of carboxyl and related chromophores is particularly diagnostic of polysaccharide conformation and interactions[9,13–15].

N.m.r. relaxation represents a powerful probe of polymer flexibility[9,16,17], being extremely rapid for rigid molecules, but slower for flexible coils, where thermal motions interfere with exchange of energy. Relaxation may be measured (*a*) directly by following the exponential decay of magnetisation, measurement of the spin–spin relaxation time (T_2) being particularly convenient; or (*b*) indirectly by high-resolution n.m.r. spectroscopy, as linewidth is inversely related to relaxation time. Thus, small molecules moving freely in solution show sharp n.m.r. spectra, whereas, for the solid state, linewidths are so great that no high-resolution spectrum can be detected. The

predominant T_2 value for typical random-coil polysaccharides is ~ 50 msec, giving high-resolution peaks which, although broader than for monosaccharides, are still clearly discernible. By contrast, rigid molecular conformations, such as the native carrageenan double-helix structure, show T_2 values of $\sim 50\ \mu$sec[18], and the corresponding high-resolution linewidth is so great that all peaks are completely flattened into the baseline. Thus, the decay of high-resolution n.m.r. spectra can be used to monitor the development of conformations of restricted mobility.

EXPERIMENTAL

Materials. — All *Arthrobacter* exopolysaccharide samples were gifts from Dr. Allene Jeanes. *Xanthomonas phaseoli* extracellular polysaccharide (NRRL B1460) was cultured and isolated by the method of Lesley and Hochster[19], from a cell preparation also generously donated by Dr. Jeanes. The following were commercial materials: the extracellular polysaccharides from *Xanthomonas campestris* and *Erwinia carotovora* ("Keltrol" and "Zanflo", respectively; Kelco Company Inc., Clark, N.J., U.S.A.), dextran (Koch–Light, code number 1527d; molecular weight range, 5×10^6 to 40×10^6), guar gum (purified grade, Kobenhavns Pektinfabrik, Copenhagen, Denmark), tara gum and locust-bean gum (purified materials; code numbers REX 5922 and REX 5924, respectively; Marine Colloids Inc., Rockland, Maine, U.S.A.). Other samples of locust-bean gum used for comparison were obtained from Kobenhavns Pektinfabrik, or H. P. Bulmers Ltd., Hereford, and the fraction of diminished net galactose content was prepared for us by Pierrefitte–Auby, Paris. Konjac mannan was prepared by purification of commercial food-grade konnjaku flour; the crude material (10 g) was extracted at room temperature with 50% aqueous ethanol (3×50 ml), the residue was dissolved in distilled water (4 l) with heating and stirring, and the resulting solution was clarified by centrifugation, and freeze-dried. (1→3),(1→4)-β-D-Glucan from barley was a gift from Dr. G. Bathgate.

Methods. — Analysis of polysaccharide sugars was effected by hydrolysis and g.l.c. analysis, after the method of Albersheim and co-workers[20]. Acetate analysis was effected by saponification and back titration[21]. C.d. spectra were recorded on a Cary 61 CD Spectropolarimeter, using a 10-sec integration period, and pathlengths in the range 0.1 mm to 1 cm as appropriate. Optical rotations were measured on a Perkin–Elmer 241 polarimeter, using a 10-cm cell. N.m.r. measurements were performed on a Varian XL-100 spectrometer operating in the Fourier-transform mode and on a Bruker SXP spectrometer operating at 60 MHz, using techniques identical to those detailed in an earlier paper[16]. Dynamic rigidity and viscosity measurements were performed on an R18 Weissenberg Rheogoniometer, using a 5-cm diameter cone and plate configuration, with a cone angle of 2 degrees, and an oscillatory amplitude of 100 μm. Rotational viscosity measurements were made on a Haake Rotovisco, using an MV1 rotor assembly. Gel-melting and -setting temperatures were determined by visual observation of the movement of stainless-steel ballbearings of 2-mm diameter. Gel strength was measured on an Instron Universal Materials Tester.

RESULTS AND DISCUSSION

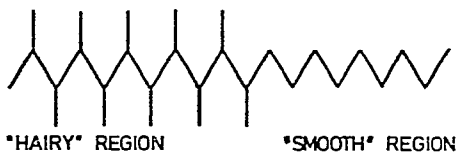
Chain–chain and chain–solvent interactions. — Association of polysaccharide chains may give rise to various effects, determined by the strength, number, and nature of the interchain junctions. Dilute solutions of random-coil molecules mark one extreme, where intermolecular interactions are negligible and the polymer is fully hydrated, while the other extreme is represented by the solid state of structurally regular, linear molecules, such as cellulose, intimately packed in a sterically regular, chain conformation. The gel state shares features with both of these extremes, and has (*a*) interchain junctions formed by association of long, structurally and conformationally regular, chain segments, as in the solid state, and (*b*) non-associated regions that are conformationally irregular and extensively hydrated, and serve to solubilise the gel network[22,23]. Thus, a critical requirement for a gelling material may be sufficient flexibility and structural irregularity to prevent complete association and precipitation, as well as the more obvious need for sufficient rigidity and regularity to allow stable, co-operative, interchain junctions to form. At polymer levels below these required for gelation, polymer–polymer interactions may be detected simply as an increase in solution vicosity.

The association of polysaccharide chains may be promoted in a number of ways. Firstly, since chain–chain association is in competition with chain–solvent association, a lowering of water activity may promote interchain binding. This effect may be achieved by addition of a hydrophilic molecule of low molecular weight, which binds water in competition with the polymer, *e.g.*, high concentrations of sucrose in pectin gelation. Association is also facilitated by decreasing interchain repulsions. Thus, chain–chain interactions of acidic polysaccharides may be promoted by lowering the pH to suppress ionisation, and increasing the ionic strength to further decrease electrostatic repulsion between the chains.

A further way of inducing interchain association is by use of a freeze–thaw cycle. On freezing a polysaccharide solution, ice formation progressively raises the effective polymer concentration in the residual unfrozen solution, with consequent promotion of association[24]. Normally, interchain junctions formed in this way dissociate on thawing. However, where the barrier to spontaneous association in solution is kinetic rather than thermodynamic, junctions once formed may persist. A practical exploitation of the effect is in the purification of lichenin, where successive freeze–thaw cycles are used to isolate the polysaccharide[25]. In the present work, we have observed almost complete precipitation of a (1→3),(1→4)-β-D-glucan from barley after a single freeze–thaw cycle.

Plant polysaccharides. — *Galactomannans.* The galactomannans form a family of seed-reserve polysaccharides[6], based on a (1→4)-linked β-D-mannan backbone solubilised by substitution with (1→6)-linked α-D-galactosyl stubs, which are present to various degrees in different galactomannans. As expected, the tendency to interchain association decreases with increasing substitution. In this work, we have studied four galactomannans. Locust-bean gum, from *Ceratonia siliqua*, has a ratio of

mannose to galactose of ~3.5:1, the galactose substituents being clustered mainly in blocks of ~25 residues[26,27] ("hairy regions") interspersed by longer regions of essentially unsubstituted mannan-backbone ("smooth regions"), as illustrated schematically in Fig. 1. Individual molecules within a particular sample of locust-bean gum may be substituted to different degrees, the least-substituted molecules showing the greatest tendency to association and precipitation. Thus, differential solubility may be used to fractionate locust-bean gum into samples having different ratios of mannose to galactose. Tara gum, which is extracted from the seeds of *Caesalpinia spinosa*, has a mannose-to-galactose ratio of ~3:1, and although detailed structural evidence is not yet available, its behaviour is so similar to that of fractions of locust-bean gum of high galactose content that a block structure seems likely. However, periodate-oxidation studies[28] indicate that guaran, the principal polysaccharide of guar gum from *Cyamopsis tetragonolobus*, does not have a block structure, and recent evidence[27] indicates a regular, alternating structure. It is perhaps significant that, in the regular, two-fold, solid-state conformation of the mannan backbone, a molecule of this structure would display a "smooth" side and a "hairy" side, as shown in Fig. 2. Although commercial samples of guar gum show variations in composition, in general they have mannose-to-galactose ratios of rather less than 2, and can be fractionated as described above for locust-bean gum[2]. This therefore suggests the presence of significant amounts of a material that does not have the proposed, regular, tri-saccharide repeating-structure, but perhaps has a block-like composition.



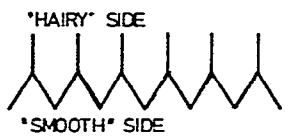


Fig. 1. Galactomannan block structure. In this schematic representation, the mannan backbone is shown in the regular, solid-state, two-fold conformation.

Fig. 2. Guaran alternating structure. Adoption by the mannan backbone of the typical, solid-state, two-fold conformation would cluster the galactose substituents on one side of the main chain, as indicated schematically.

Gradation of galactomannan structure is directly reflected in chain–chain interactions. Thus, on freezing and re-thawing after 24 h, guar gum shows no evidence of association, tara gum at concentrations of $\geqslant 0.75\%$ forms weak gels that break down on heating to 30°, and locust-bean gum forms a weak, but cohesive, gel network at concentrations as low as 0.5%. Freeze–thaw galactomannan gels show no true

melting point, but on heating, the structural integrity may be lost over a fairly narrow range of temperature, leaving a suspension of gel particles. Thus 1% gels of locust-bean gum break down at 64–67°, 0.75% gels at 60–65°, and 0.5% gels at 50–55°. At 0.25%, association of locust-bean gum is limited to gel-island formation on freezing and thawing. Freeze–thaw treatment of a preparation of locust-bean gum freely soluble in hot water, but less soluble in cold water, and whose ratio of mannose to galactose is ~4.5:1, gives extremely firm, rubbery gels, which, at higher concentrations, may be broken down only on autoclaving. Moreover, a 2% solution kept at ambient temperature gradually develops structure, until, after ~3 days, a cohesive gel is formed. This process is accompanied by a gradual shift in optical rotation, indicating changes in net conformation of the polymer backbone. We have observed similar behaviour for unfractionated locust-bean gum, but much higher concentrations are required. Indeed, certain samples of locust-bean gum may be stored for protracted periods at 2°, at concentrations as high as 8%, with no evidence of structure formation. Galactomannan gels are unstable, and on a second freeze–thaw cycle may lose up to 50% of their water content by syneresis.

A similar correlation of bulk properties with galactomannan composition is evident on inducing intermolecular association by lowering the water activity. Thus, in 50% aqueous ethylene glycol, locust-bean gum forms a very weak, but cohesive, gel at concentrations down to 0.2%, whereas tara gum requires a concentration of 0.5% for gelation, and guar gum precipitates (*cf.* Ref. 29). At higher concentrations, locust-bean and tara gums both give good, firm gels, that for locust-bean gum being the stronger. Closely similar results are observed with glycerol (50% v/v), or sucrose (60% w/v). On the basis of the above evidence, we therefore propose a model (Fig. 3) for galactomannan gel structure, in which chain–chain association is by aggregation of "smooth" mannan regions in a regular, ribbon-like, two-fold conformation, with "hairy" regions serving to solubilise the network. The lack of such junction-terminating sequences in guaran is consistent with its precipitation rather than gelation, as discussed above.

Konjac mannan. Konjac mannan is a β-(1→4)-linked linear co-polymer of D-glucose and D-mannose, derived from the tubers of *Amorphophallus konjac*. The backbone of this polymer is thus very similar to cellulose, differing only in the configuration at C-2 of the mannose residues, and might therefore be expected to be extremely insoluble. Solubility is conferred, however, by the presence of *O*-acetyl substituents which, as in cellulose acetate, presumably hinder chain packing, and by providing additional conformational entropy represent a further barrier to association. Saponification analysis shows an acetate content of 3.7%, corresponding to approximately one acetyl group per six glycosyl residues. A residual tendency to chain–chain aggregation is shown, however, by the rheological behaviour of aqueous solutions. At 10% concentration, konjac mannan forms a viscoelastic dough or "putty", which can be moulded, heals after mechanical damage, flows over long periods, but, on a shorter timescale of relaxation, will bounce like rubber. Similar behaviour persists down to 5% concentrations. Below this level, moulding is no longer possible, but

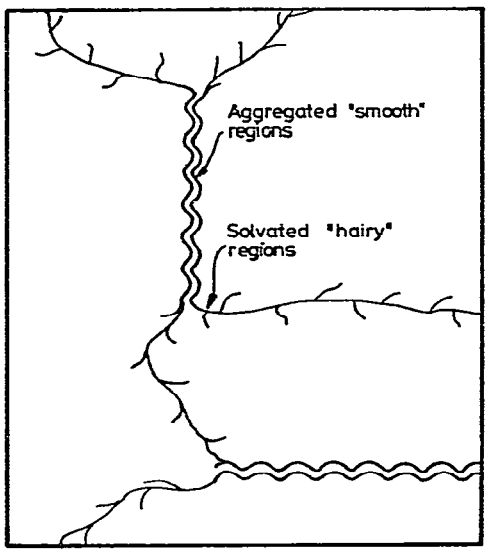


Fig. 3. Proposed structure for galactomannan gel. Unsubstituted mannan-backbone regions associate as in the solid state, to form interchain junctions, while substituted regions are extensively hydrated, as in solution, and prevent complete precipitation.

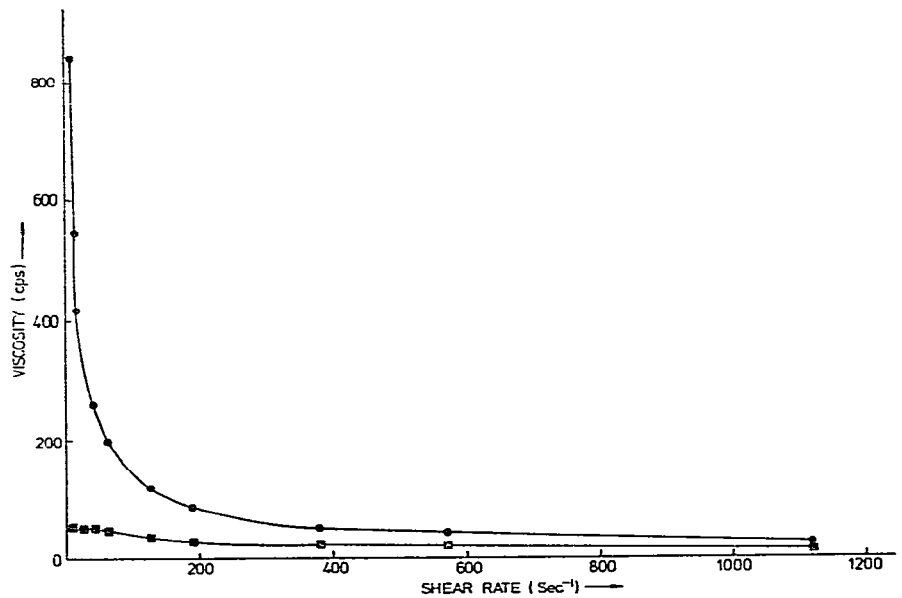


Fig. 4. Conformation dependence of non-Newtonian viscosity in xanthan solutions. At 25° (●), in the native rod-like conformation, xanthan (1% w/v solution of *X. campestris* polysaccharide) shows a marked enhancement of viscosity at low shear, which is lost at 85° (■), where the molecule is in the coil form.

distinct putty-like properties are observed as low as 2%, while solutions of even lower concentration still display marked viscoelasticity. Konjac mannan "putties" become more mobile on heating, but show no distinct melting point.

Bacterial polysaccharides. — *Xanthomonas campestris* extracellular polysaccharide (xanthan), which has found technological application, has a cellulose backbone substituted on every second residue with a charged trisaccharide side-chain[30,31]. In the solid state, the trisaccharide stubs align with the polymer backbone to produce a rigid, rod-like structure. This structure persists in solution under most circumstances, and is melted out only under conditions of elevated temperature and low ionic strength[3,4,6,32,33]. The practical importance of xanthan rests mainly on its extreme shear thinning, and its emulsion-stabilising and particle-suspending abilities, all of which point to intermolecular association in solution. We suggest that this structure is built up by the alignment and tenuous association of rigid molecules in solution[3]. Thus, in Fig. 4, we compare the shear dependence of xanthan viscosity at ambient temperature, in the native molecular conformation, and at an elevated temperature where the molecule has adopted a normal coil conformation. At high rates of shear, the difference in viscosity is no greater than would be expected on heating a solution of a random-coil polymer. At low rates of shear, however, the low-temperature solution shows a massive increase in viscosity, consistent with the

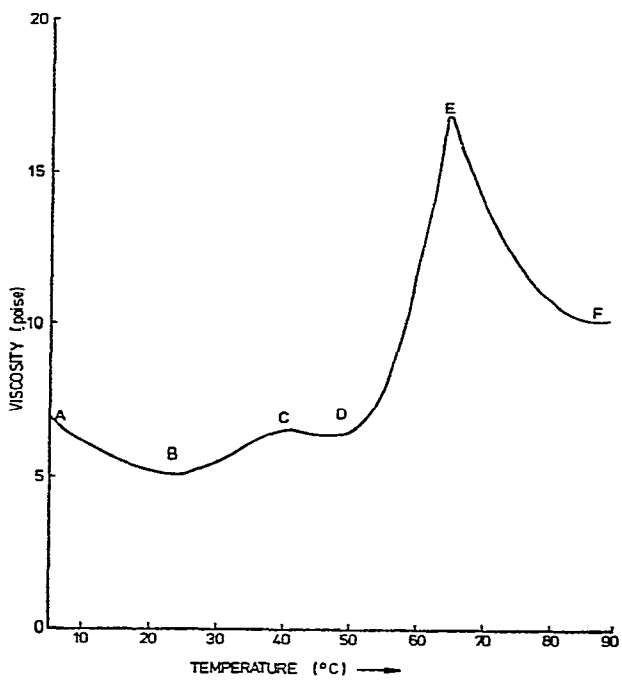


Fig. 5. Temperature profile of xanthan solution viscosity. A constant shear rate of 7 sec^{-1} was used. Xanthan (*X. campestris*) concentration was 1% w/v, with no added salt.

presence of weak, intermolecular associations that are destroyed at high rates of shear, and have no counterpart at high temperature. Fig. 5 shows the temperature profile of the viscosity of xanthan solution at a constant rate of shear. The curve shows two regions of anomalous increase of viscosity on heating. The higher region (D–E) corresponds to the breakdown of the native conformation, as characterised by the spectroscopic methods discussed in the Introduction. Our earlier work has shown that intrinsic viscosity decreases sharply on heating through the helix–coil transition, consistent with the greater radius of gyration of a rigid rod. We therefore attribute the viscosity increase at higher concentrations to intermolecular entanglement, which is likely to occur more readily between flexible coils than between stiff rods. Once in the coil form, the molecule shows the normal decrease in solution viscosity on heating (Fig. 5, E–F). We suggest that the first anomalous rise in viscosity over the region B–C marks the breakdown of rod–rod aggregates, analogous to the disruption of galactomannan ribbon–ribbon associations on heating. Fig. 6 shows the concomitant, anomalous increase in dynamic viscosity and rigidity, as monitored by oscillatory measurements, which have the advantage of being less likely to disrupt tenuous intermolecular associations. Further evidence of the alignment and aggregation of xanthan rods comes from the observation[34] of massive birefringence effects in concentrated solutions of the polymer.

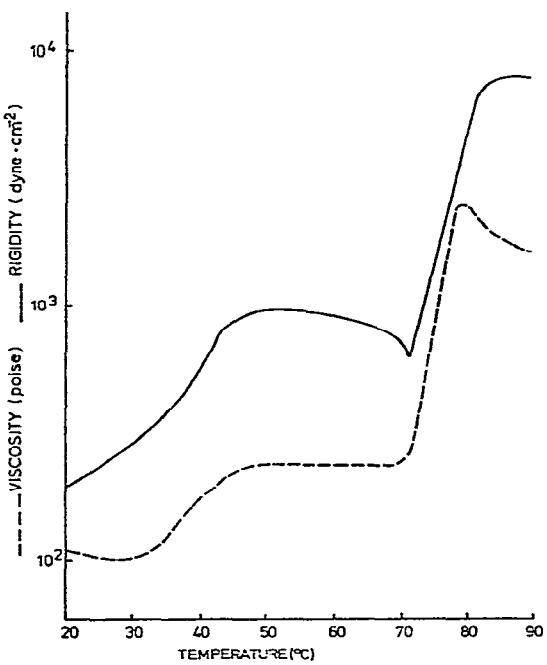


Fig. 6. Temperature dependence of the dynamic rigidity and viscosity of xanthan solutions. A fixed oscillatory frequency of 1.57 sec^{-1} was used. Xanthan (*X. campestris*) concentration was ~1.5% w/v, with no added salt.

Xanthomonas phaseoli yields an extracellular polysaccharide whose structure has not as yet been fully elucidated. Preliminary evidence[19,35] indicates a marked similarity to the exopolysaccharide from *X. campestris*. The c.d. spectrum (Fig. 7) is consistent[13] with the known presence of D-glucuronic acid as a major constituent of the polymer. As shown in Fig. 8, the c.d. peak height shows a marked discontinuity on heating, indicative of a co-operative change in conformation. This view is confirmed

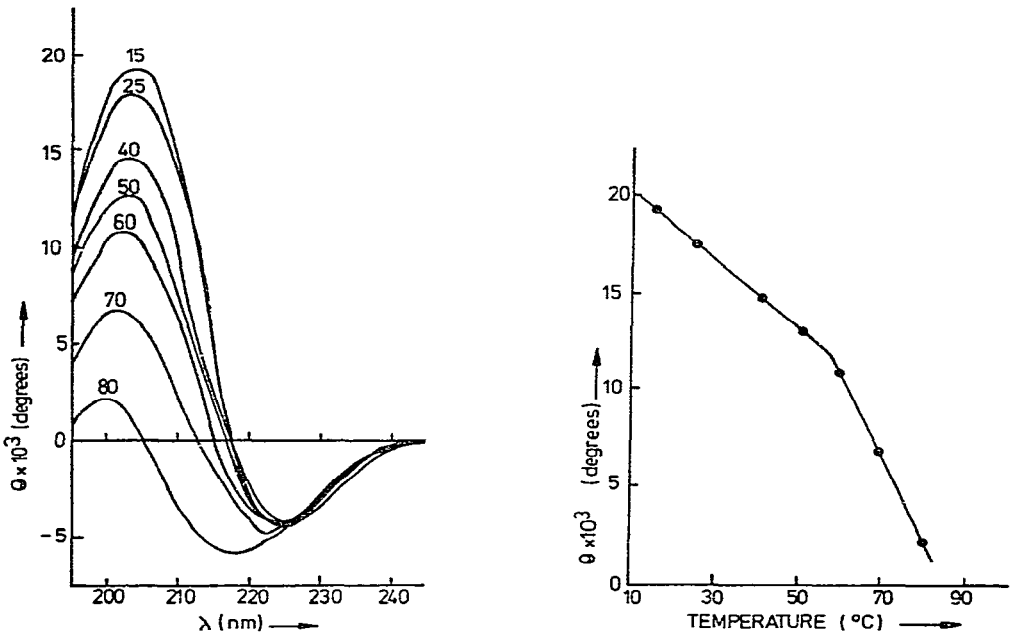


Fig. 7. Circular dichroism of *Xanthomonas phaseoli* extracellular polysaccharide. Temperature (degrees) is indicated above each spectrum (0.5% w/v concentration; 1-mm pathlength).

Fig. 8. C.d.–temperature profile for *Xanthomonas phaseoli* extracellular polysaccharide. Temperature dependence of the maximum ellipticities of the spectra in Fig. 7 is shown. The observed discontinuity is indicative of the onset of a co-operative conformation change.

by single-wavelength measurements of optical rotation (Fig. 9), which show the sigmoidal temperature course characteristic of an order–disorder transition. The optical-rotation shift on cooling through the transition region is negative, opposite in sense to the observed c.d. changes. We therefore conclude that the optical-rotation behaviour cannot be explained in terms of restricted mobility of a pendent chromophore, but has its origins in the vacuum u.v., where transitions of the polysaccharide backbone are known to occur[36].

The full primary structure of the samples of *Arthrobacter* extracellular polysaccharide has not yet been determined, but preliminary evidence of composition is summarised in Table I. Aqueous solution of the extracellular polysaccharide from *Arthrobacter stabilis* (NRRL B3225) show thermally reversible, gelation behaviour,

which is accompanied by a sigmoidal transition in optical rotation (Fig. 10), indicating that gelation occurs by co-operative association of chains into conformationally regular, junction zones. The optical-rotation transition persists at concentrations well below the minimum necessary for gelation, confirming that it has a genuine molecular origin, rather than being simply a bulk, optical artefact of gelation. Deacetylation of the polymer shifts the transition to lower temperatures by $\sim 10°$ (Fig. 11), suggesting

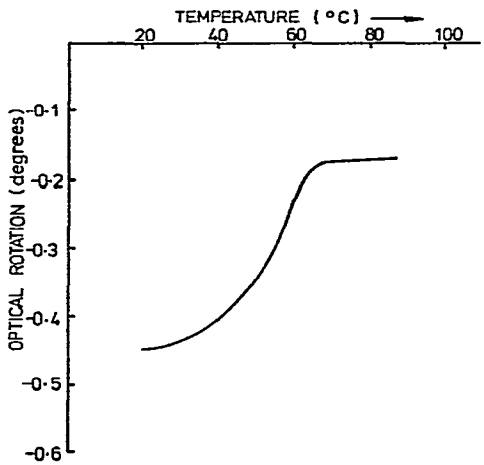


Fig. 9. Optical rotation–temperature profile for *Xanthomonas phaseoli* extracellular polysaccharide (365 nm; 10-cm pathlength; 1% w/v polysaccharide concentration; no added salt).

TABLE I

COMPOSITION OF *Arthrobacter* EXTRACELLULAR POLYSACCHARIDES

Synthesising bacterium	*NRRL strain*	*Constituent sugars (molar ratios)*	*Substituents (%)*		*Refs.*
A. stabilis	B 3225	Glucose–galactose (2:1)	Acetate Succinate Pyruvate	5.0 3–5 5.1	46
A. viscosus	B 1973	Glucose–galactose–mannuronic acid (1:1:1)	Acetate	25	44,45
A. viscosus sp.n.	B 1797	Glucose–galactose–glucuronic acid (3:3:1)	Acetate Pyruvate	8.0 5.5	46

that the acetate groups exert a solubilising effect antagonistic to chain–chain interactions, but do not appreciably affect the geometry of the ordered conformation. C.d. evidence further supports this interpretation, as shown in Fig. 12, where once more a sharp, sigmoidal transition is observed on plotting the temperature profile of c.d. maximum ellipticity, with deacetylation having little effect on the magnitude of the

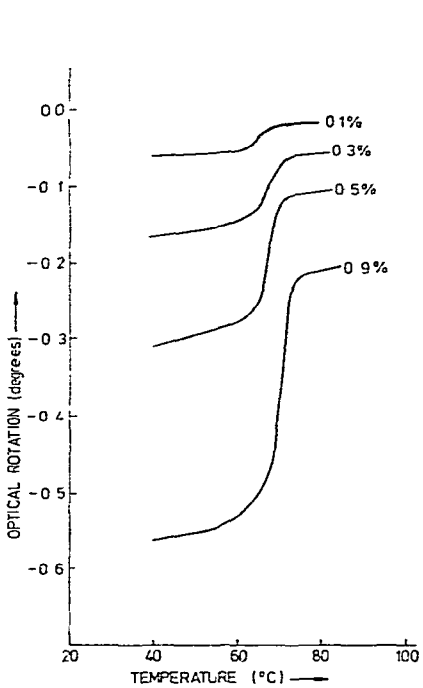


Fig. 10. Optical rotation–temperature profiles for *Arthrobacter stabilis* extracellular polysaccharide (365 nm; 10-cm pathlength; no added salt). Polysaccharide concentrations are shown on the diagram.

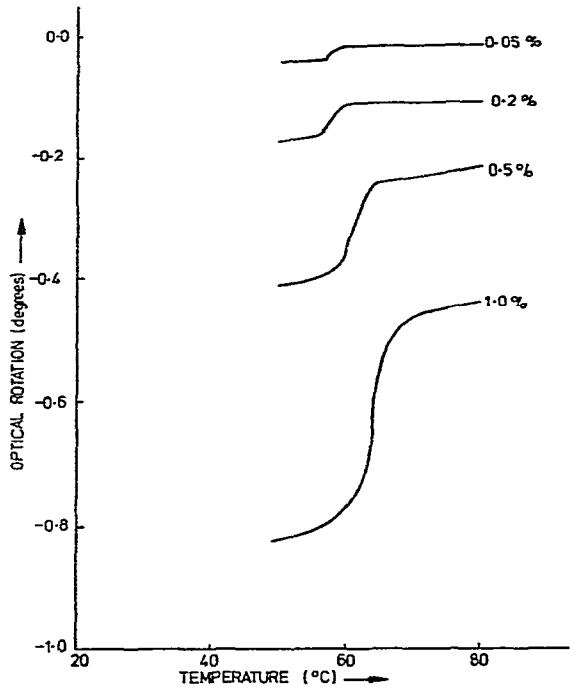


Fig. 11. Optical rotation–temperature profile for deacetylated *Arthrobacter stabilis* extracellular polysaccharide (conditions as in Fig. 10).

c.d. change but again shifting the midpoint of the transition to lower temperatures. Thus, the molecular environment of the pyruvate groups, which are the only other accessible u.v.-chromophores on the molecule, is substantially altered by adoption of the ordered conformation, whereas the acetate groups make little contribution to the c.d. In contrast, as shown in Fig. 13, acetate substituents dominate the c.d. behaviour of the extracellular polysaccharide from *Arthrobacter viscosus* (NRRL B1973). These groups, although present to over 60% of the theoretical value for complete substitution, do not appear to be particularly sensitive to changes in the conformation of the polymer backbone; in particular, the temperature profile of circular dichroism and optical rotation shows only slight inflexion on gelation (Fig. 14). Deacetylation completely eliminates gelling behaviour, and the circular dichroism (Fig. 13) is then entirely consistent[13] with the known presence of mannuronic acid as the only other accessible u.v.-chromophore in the molecule. Although the swamping effect of the acetate groups interferes with chiroptical characterisation of the gelation process, n.m.r.-relaxation evidence is extremely diagnostic. As shown in Fig. 15, no high-resolution spectrum can be detected at ambient temperature, consistent with a rigid molecular conformation. However, on

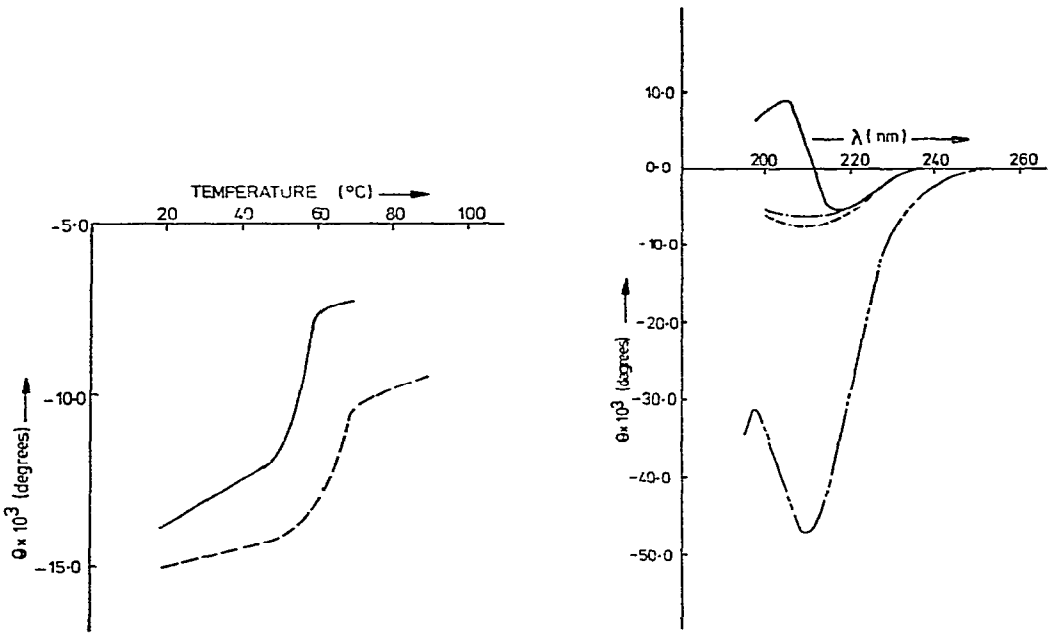


Fig. 12. C.d.–temperature profile for *Arthrobacter stabilis* extracellular polysaccharide. The temperature dependence of maximum ellipticity (see Fig. 13) is shown for both the native (– – –) and the deacetylated (———) polymer (0.5-mm pathlength; 1% w/v polysaccharide concentration; no added salt).

Fig. 13. C.d. of *Arthrobacter* extracellular polysaccharides (conditions as in Fig. 12). *A. viscosus*: native, (— — —); deacetylated, (———). *A. stabilis*: native, (– – – – –); deacetylated. (—·—).

heating to well above the melting point of the gel, a typical polysaccharide-coil spectrum emerges. As shown in Fig. 16, the exopolysaccharide from *Arthrobacter viscosus* sp. n. (NRRL B1797) shows similar evidence of existing at ambient temperatures in an ordered, native conformation, which is melted out on heating, with concomitant breakdown of gel structure.

By contrast, the extracellular polysaccharide from *Erwinia carotovora*[37] shows a normal-polymer high-resolution spectrum at all temperatures (Fig. 17), indicative of a normal coil conformation. Similarly, dextran, the exopolysaccharide of *Leuconostoc mesenteroides* (and other related strains), which is a (1→6)-α-D-glucan with (1→3)-α-D branchpoints[38], shows no evidence of adopting specific, restricted conformations in solution, which is entirely consistent with its highly branched, flexible, primary structure.

Interactions between unlike chains. — Until recently[2], synergistic interactions between different polysaccharides in solution have generally been ascribed to competition for solvent by dissimilar polymer molecules[1]. We have characterised a particularly striking example of this type of interaction. As already discussed, gelation of locust-bean gum can be induced by lowering of the water activity. Thus, in the

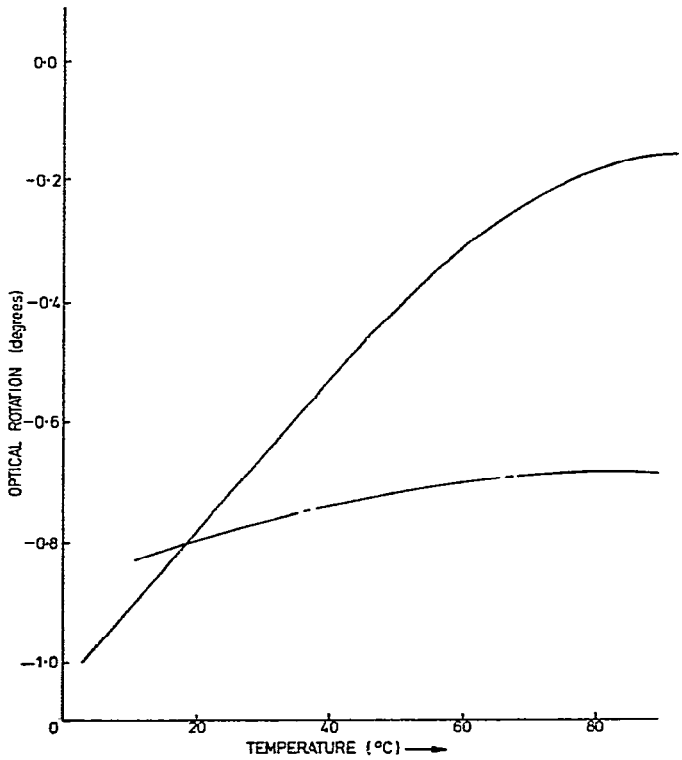
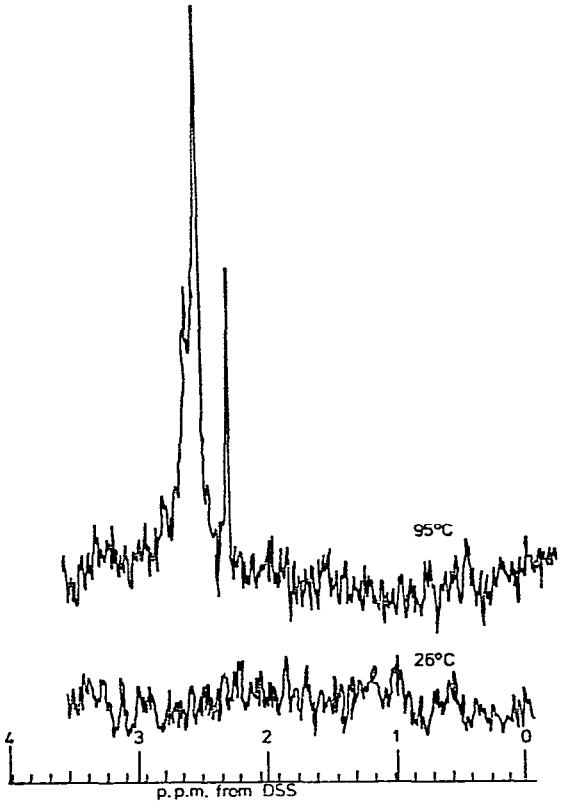


Fig. 14. Optical rotation–temperature profile for *Arthrobacter viscosus* extracellular polysaccharide (365 nm; 10-cm pathlength; no added salt). Native, (———); deacetylated (— — —).

presence of 60% (w/v) sucrose, 0.5% locust-bean gum develops a weak, but cohesive, gel structure on low-temperature storage (typically 2° for 1 week). Under similar conditions, dextran solutions show no evidence of the development of structure, but remain as mobile liquids. In combination, under conditions of low water-activity, however, the two polysaccharides show marked enhancement of gel structure. Thus, on mixing solutions of dextran and locust-bean gum, such that the combined solution is 60% w/v in sucrose and 0.5% w/v in each of the polysaccharides, a thick, viscoelastic putty is formed immediately. On holding at low temperature as before, a firm gel develops, with rigidity characteristics that are an order of magnitude greater than for galactomannan alone. Typically, the rigidity of such a gel, close to its natural frequency of oscillation, was ~ 2400 dynes.cm^{-2} (*cf.* 120 dynes.cm^{-2} for locust-bean gum alone). Doubling of the galactomannan concentration to the same total polysaccharide level as in the mixed system has far less effect (typically a doubling of gel strength) than the addition of the non-gelling dextran.

Freeze–thaw evidence indicates the origin of this behaviour. Thus, on freezing and rethawing a dilute, mixed solution of dextran and locust-bean gum, a precipitate is formed, which contains only galactomannan of the same sugar composition as the



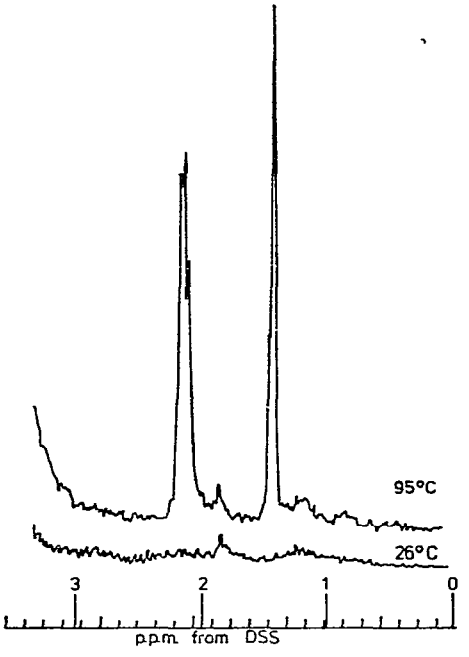


Fig. 15. High-resolution p.m.r. spectrum for *Arthrobacter viscosus* extracellular polysaccharide. Collapse of the high-resolution spectrum on cooling indicates adoption of a rigid conformation.

Fig. 16. High-resolution p.m.r. spectrum for *Arthrobacter viscosus* sp. n. extracellular polysaccharide.

locust-bean gum. The absence of dextran in the precipitate, and the lack of specificity in the block composition of the galactomannan precipitated and retained in solution, both indicate non-association of dextran and locust-bean gum molecules in solution. Rather, they suggest an incompatibility of the two polymers with exclusion of one from the domain of the other, thus considerably increasing the effective concentration of both. The greater rigidity and ease of packing of locust-bean gum molecules explains why it, rather than dextran, should be expelled from solution in the freeze–thaw situation.

The concept of incompatibility has also been invoked[39] to explain the gelation of locust-bean gum with xanthan[39,40]. We now offer evidence to suggest that a very different mechanism is in operation. Xanthan alone does not gel at any concentration, although, as previously discussed, there is evidence of tenuous intermolecular association in solution, leading to marked shear thinning. In combination with locust-bean gum, however, firm, mixed gels are formed at low concentrations of total

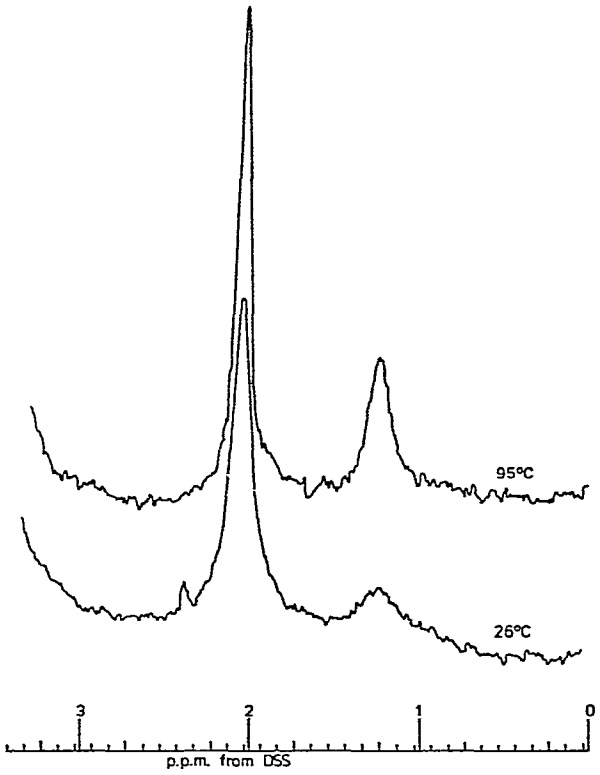


Fig. 17. High-resolution p.m.r. spectrum for *Erwinia carotovora* extracellular polysaccharide.

polysaccharide. These are true gels, which do not flow, and do not recover from mechanical damage. In contrast to the dextran–locust-bean gum system, no lowering of water activity is required, and structure forms instantaneously, rather than developing slowly on standing. Furthermore, the gels are thermally reversible, and show sharp melting and setting behaviour over a narrow range of temperature. As illustrated in Table II, gel setting and melting points increase with increasing content of total polysaccharide, but show little dependence on the relative levels of the two polymers. Although, as shown, substitution of tara for locust-bean gum does not significantly alter the temperature course of gelation, substantially weaker gels are formed, whereas locust-bean gum of diminished galactose content gives much stronger gels, and guar does not gel at all. There is strong evidence, however, that this behaviour reflects the lack of block structure in guar, and its consequent inability to cross-link a gel network, rather than lack of interaction with xanthan.

Fig. 18 shows the optical rotation–temperature profile of xanthan and tara gum alone, and of a gelling mixture of the two. The optical rotation of tara gum is virtually independent of temperature, consistent with a coil conformation, whereas xanthan shows a sharp, sigmoidal increase in optical activity on adoption of the low-temper-

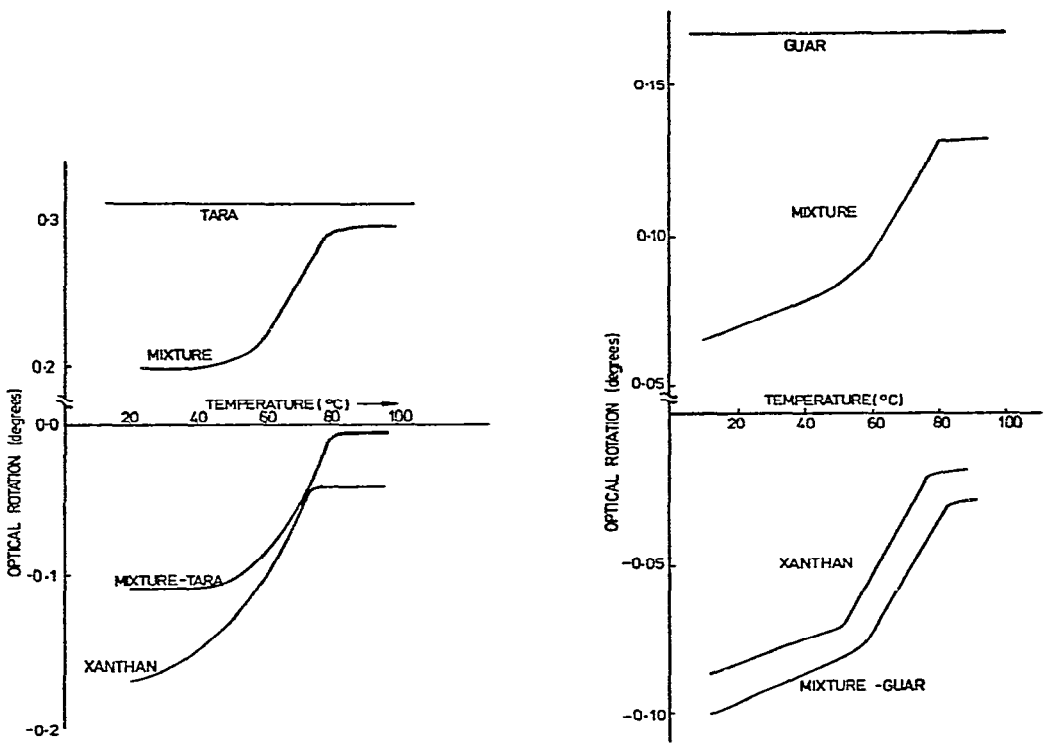


Fig. 18. Conformational changes during galactomannan gelation with xanthan. Optical rotation evidence shows the maintenance and stabilisation of xanthan native conformation in mixed gels (365 nm; 10-cm pathlength; 0.35% w/v tara, 0.5% w/v xanthan from *X. campestris*; no added salt).

Fig. 19. Interaction of guar gum with xanthan. Optical rotation shows stabilisation of the xanthan native conformation in the presence of guar (365 nm; 10-cm pathlength; 0.5% w/v guar, 0.5% w/v xanthan from *X. campestris*; no added salt).

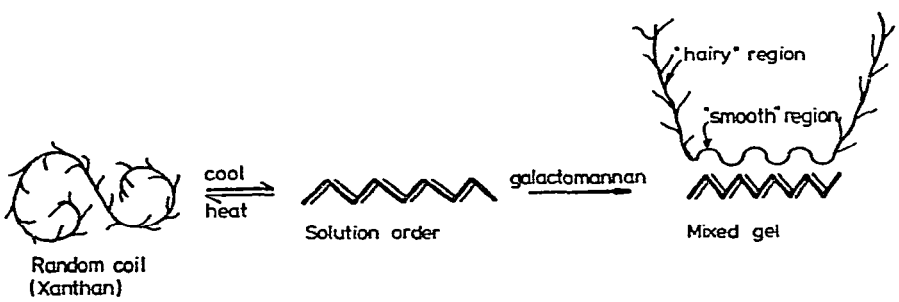


Fig. 20. Molecular origin of xanthan interaction with galactomannans; the mixed junctions may be formed by co-operative association of mannan-backbone regions with the xanthan native structure, as schematically indicated.

ature, ordered conformation previously discussed. The order–disorder transition is still clearly in evidence in the synergistic system, but shifted to higher temperature by $\sim 10°$, thus clearly suggesting stabilisation of the xanthan helix by interaction with galactomannan. The temperature profile below the gel point in the mixed system is significantly flatter than that for xanthan alone, perhaps indicating inhibition of xanthan aggregation by network constraints. Xanthan–locust-bean gum gels show exactly analogous behaviour, but measurements of optical activity below the gel point are irreproducible, presumably due to bulk artefacts of gel strain. The weaker gels of tara gum, however, give no evidence of any such complications, and show completely reproducible optical-rotation behaviour. Moreover, the synergistic properties of xanthan from *X. campestris* and *X. phaseoli* appear to be virtually identical. As shown in Fig. 19, guar gum also stabilises the xanthan helix, again by $\sim 10°$. In this case, however, the optical rotation profile at low temperature is scarcely affected, consistent with the absence of gel-network formation. The marked dependence of gel strength on galactomannan structure once more implicates unsubstituted "smooth" mannan-backbone regions in junction formation, and we therefore propose the schematic model for xanthan–galactomannan gelation outlined in Fig. 20. This scheme closely parallels our earlier conclusions[2] on the nature of the synergistic interactions of galactomannans with agarose and carrageenans.

TABLE II

XANTHAN–GALACTOMANNAN GELATION TEMPERATURES

Total polysaccharide concentration (%)		*Xanthan–galactomannan ratio*		
		1:1	*1:2*	*1:3*
Locust-bean gum	2%	39–41	40–41	40–41
	1%	33–36	34–36	36–37
	0.5%	29–32	29–31	29–31
Tara gum	1.4%	35–36	35–37	35–38

The first figure in each case is the setting point of the gel (degrees), and the second the melting point.

Rheological studies provide further evidence of the interaction between guar and the xanthan native structure in solution. Under conditions of high shear, no interaction is detected. Use of oscillatory techniques, however, allows tenuous intermolecular associations to be preserved, and a marked enhancement of solution viscosity is then observed (Fig. 21). Measurements of dynamic rigidity provide even stronger evidence of interaction, the rigidity of mixed xanthan–guar solutions being an order of magnitude greater than those of either component alone at the same total level of polysaccharide (Fig. 22). The success of this approach to the detection of intermolecular interactions prompted us to apply the same methods to the study of

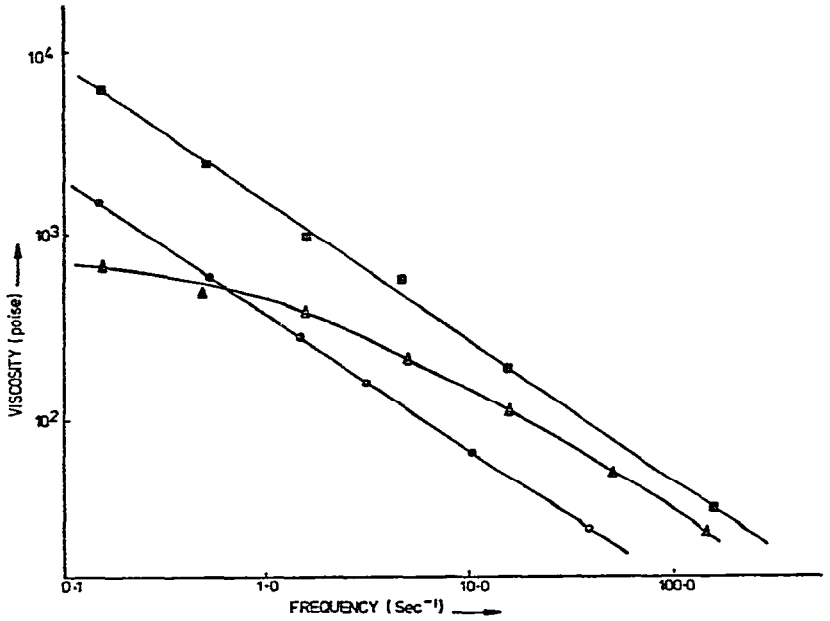


Fig. 21. Viscous interaction between xanthan and guar: ●, xanthan from *X. campestris*; ▲, guar gum; ■, interacting 1:1 mixture (total polysaccharide concentration, ~1.5% w/v).

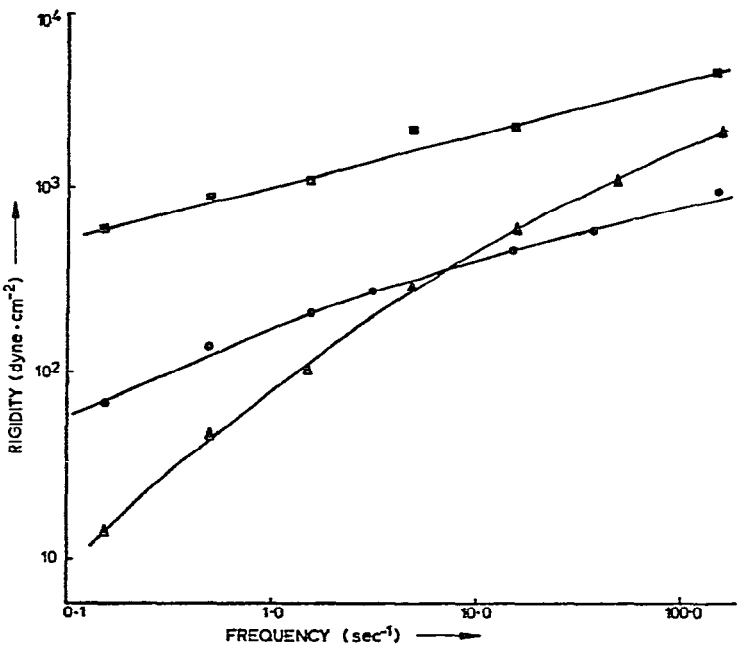


Fig. 22. Enhancement of dynamic rigidity on interaction of xanthan with guar (symbols and conditions as in Fig. 21).

interactions of other bacterial gums with plant polysaccharides. To optimise the possibility of interaction, we have used the low-galactose fraction of locust-bean gum previously discussed, which approaches the highest level of unsubstituted glycan backbone compatible with solubility, and shows far stronger interaction with xanthan than any other galactomannan studied. However, as shown in Fig. 23, dynamic viscosity and rigidity measurements show no evidence of interaction with the exopolysaccharide from *Erwinia carotovora*. In view of the lack of ordered secondary structure, the failure of this material to interact with mannan is, perhaps, not unexpected.

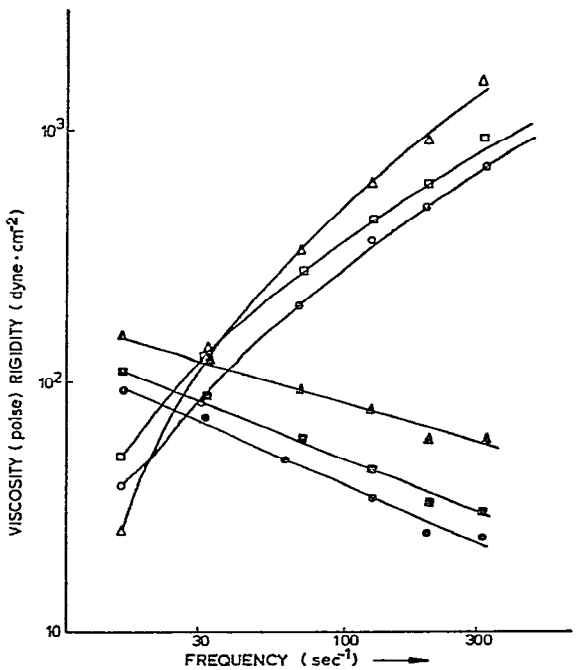


Fig. 23. Dynamic viscosity and rigidity show no interactions between galactomannans and *Erwinia carotovora* extracellular polysaccharides (total polysaccharide concentration, ~1.5% w/v, with no added salt): ●, ○, bacterial polysaccharide; ▲, △, galactomannan; ■, □, 1:1 mixture; filled symbols show viscosity, and open symbols show rigidity.

Similar lack of interaction, however, is shown by the *Arthrobacter* polysaccharides, whose rigid, native conformations have been demonstrated. This situation is illustrated in Fig. 24 for *A. stabilis* exopolysaccharide, but closely similar results were obtained for both the *A. viscosus* materials studied. The concentrations of bacterial polysaccharide used (higher than those needed to demonstrate the xanthan–guar interaction) are just insufficient for gelation, but spectroscopic evidence clearly establishes the presence of an ordered conformation. Preliminary investigations[5], using gelling concentrations of *Arthrobacter* polysaccharide, had shown evidence of

viscous interactions with concentrated solutions of locust-bean gum. Our present work, however, suggests that such interactions are probably non-specific bulk effects, perhaps analogous to the locust-bean gum–dextran incompatibility discussed above, rather than a result of specific intermolecular interactions.

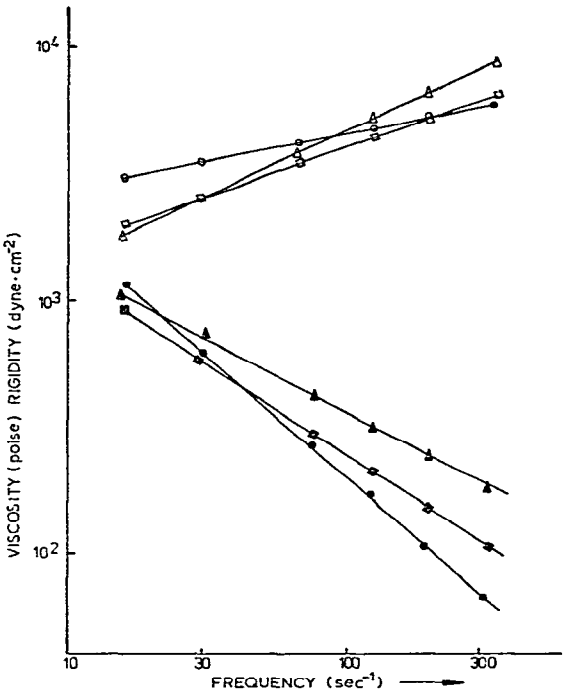


Fig. 24. Dynamic viscosity and rigidity show no interaction between galactomannans and *Arthrobacter stabilis* extracellular polysaccharide; closely similar profiles were obtained with polysaccharides from *A. viscosus* species (symbols and conditions as in Fig. 23).

The chemical composition of the plant polysaccharide also appears to affect critically the strength of interaction. Comparison of the melting and setting temperatures for xanthan gelation with locust-bean and tara gums (Table II) indicates that the nature of the polymer backbone largely determines the energy of individual junctions, although the number of such junctions must clearly depend on block structure. It is therefore of particular interest to find that konjac mannan, whose polymer backbone contains both glucose and mannose also forms mixed gels with xanthan. Indeed, the interaction appears far stronger than with galactomannans. Thus, a mixed gel of 0.25% of xanthan and 0.25% of konjac mannan melts at ~63°. By comparison, the highest melting-point observed with galactomannan is 41°. Furthermore recognisable gels are formed at total polysaccharide levels as low as 0.02%. We believe this to be the lowest gelling-concentration so far observed for a

carbohydrate system, five times lower than the threshold concentration for agarose gelation[2,41].

Comparison of galactomannan and glucomannan synergism with other ordered polysaccharides establishes that this enhancement of gel structure is a consequence of specific interaction with xanthan, rather than a general property of konjac mannan. Thus, 0.05% of agarose (a non-gelling concentration) forms cohesive gels with 0.1% of locust-bean gum, whereas 0.25% of konjac mannan is required for gelation. Moreover, deacetylation of xanthan marginally improves its gelation behaviour with locust-bean gum. With konjac mannan, however deacetylation reduces the gel-melting and -setting temperatures by $\sim 20°$.

CONCLUSIONS

It has recently been shown[3] that the unusual, and industrially valuable, rheological behaviour of xanthan gum is due to its maintaining a stiff, rod-like, native conformation in solution. Theoretical treatment of the packing of rigid rods[42] shows that alignment in solution may become a geometric necessity at relatively low concentrations, and we have suggested that such alignment builds up structure in xanthan solutions. Similar considerations may apply to the association of other rigid molecules, such as derivatised cellulose[24,43]. Furthermore, the loss of entropy on association of rigid molecules is slight, and may be easily outweighed by energetically favourable, chain–chain interactions, in contrast to flexible polymers, where conformational freedom in solution is a powerful barrier to interchain association. It is therefore perhaps not surprising that the most-striking examples of polysaccharide synergism all involve the interaction of rigid molecular conformations, such as the agarose, carrageenan, or xanthan native conformations, with stiff, unsubstituted chains.

In the light of the evidence presented here, we therefore distinguish different types of interaction between polysaccharides: mutual exclusion of incompatible coils, and alignment and interaction of stiff structures of compatible geometry to form mixed aggregates, which may or may not be reinforced by highly specific, intermolecular associations, such as in the interaction between the native xanthan structure and a plant (1→4)-β-D-glycan. Our present conclusion that other rigid, ordered, bacterial exopolysaccharides show no comparable interaction provides further evidence of the specificity of this association, and lends credence to our earlier suggestion of a biological role in pathogen–substrate recognition.

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