ELSEVIER   0144-8617(95)00098-4

Screening for synergistic interactions in dilute polysaccharide solutions

F.M. Goycoolea,[a] E.R. Morris[a]* & M.J. Gidley[b]

[a]Department of Food Research and Technology, Cranfield University, Silsoe College, Silsoe, Bedford MK45 4DT, UK
[b]Unilever Research, Colworth Laboratory, Sharnbrook, Bedford MK44 ILQ, UK

A simple viscometric approach has been used to screen for binding interactions between different polysaccharides in very dilute solution where exclusion effects should be negligible. The method involves preparing stock solutions to approximately the same, low, viscosity ($\eta_{sp} \approx 1$), dialysing to identical ionic conditions, mixing in various proportions, and looking for departures from the initial common viscosity.

Mixtures of xanthan or de-acetylated xanthan with locust bean gum (LBG) or konjac glucomannan (KM) show massive enhancement of viscosity, as anticipated from the formation of synergistic gels at higher concentrations. However, no viscosity changes on mixing with LBG or KM were observed for other conformationally ordered bacterial polysaccharides (welan and rhamsan) or for alginate and pectin with sufficient Ca^{2+} to induce almost complete conversion to the dimeric 'egg box' form, demonstrating that conformational rigidity is not, in itself, sufficient for other polysaccharides to form heterotypic junctions with mannan or glucomannan chains.

Interactions of carrageenans with LBG appear to depend on both conformation and the extent of aggregation. Mixtures of LBG with K^+ kappa carrageenan in 100 mM KCl (which is known to promote extensive aggregation of double helices) gave erratic values for rotational viscosity and showed typical gel-like mechanical spectra under low-amplitude oscillation. Disordered carrageenans (K^+ kappa in water and lambda in 100 mM KCl) showed no evidence of interaction with LBG. Negative results were also obtained for iota carrageenan under ionic conditions believed to promote ordering without significant aggregation (100 mM KCl). However, under conditions where limited aggregation might be expected (iota carrageenan in 90 mM $CaCl_2$; Me_4N^+ kappa carrageenan in 150 mM Me_4NI), significant reductions in viscosity were observed on mixing with LBG, which may indicate some intermolecular association but without the formation of an extended network structure. Copyright © 1996 Elsevier Science Ltd.

INTRODUCTION

When two different biopolymers are present together in aqueous solution, the enthalpic interactions between chain segments of the same type are normally more favourable than interactions of one polymer with the other, thus inhibiting intimate mixing and interpenetration of unlike chains (Tolstoguzov, 1986, 1988, 1991). Such thermodynamic incompatibility and exclusion effects may cause substantial changes in the properties of the individual polymers. For example, if one component can undergo a thermally reversible disorder–order transition, the presence of the second biopolymer may promote adoption of the more

compact ordered form and thus displace the transition to higher temperature (see, for example, Kasapis & Morris, 1993). Exclusion effects are strongly dependent on concentration, becoming undetectable in very dilute solution where the individual chains are widely separated, and increasing in magnitude as the polymer concentration is raised.

At sufficiently high concentrations the mixed solutions may resolve spontaneously into two co-existing liquid phases, each enriched in one component and depleted in the other. For gelling systems, the enhanced concentration of each polymer within the phase where it predominates can give composite gels which are much stronger than would be anticipated from the properties of the individual components at their overall nominal concentrations. Enhancement of

*Author to whom correspondence should be addressed.

gel properties in mixed biopolymer systems can, however, occur in an entirely different way, by direct association of the two materials to form a coupled network.

For polysaccharide systems, the least controversial example of heterotypic association is probably the co-gelation of alginate and pectin (Toft, 1982). The extent of interaction increases as the poly-L-guluronate content of the alginate is increased, indicating binding of these sequences to the near mirror-image poly-D-galacturonate sequences in pectin. Gel formation is promoted by methyl esterification of the pectin component and by reduction in pH, both of which would be expected to facilitate interaction by suppressing electrostatic repulsion between the participating chains. In mixtures where the methyl-D-galacturonate content of the pectin and L-guluronate content of the alginate are equal, maximum gel strength is attained when the two polymers are present in equal amounts (Toft et al., 1986), consistent with the stoichiometric relationship that would be expected for direct binding. Also, the changes in circular dichroism observed on acidification of mixed solutions of alginate and pectin are appreciably different from those anticipated from the behaviour of the individual polymers in isolation (Thom et al., 1982), again indicative of heterotypic association.

However, most research interest in apparent binding between different polysaccharides has centred on the 'synergistic' gelation of galactomannans and related ß-D-glycans with xanthan or with gelling polysaccharides in the agar/carrageenan series (Dea & Morrison, 1975; Dea & Rees, 1987). Two common features of these systems are that the plant polysaccharides all share the same (1 → 4)-diequatorial linkage geometry (so that their ordered structures in the condensed phase are flat, extended ribbons), and that the other components all undergo thermoreversible disorder–order transitions under hydrated conditions. It is also well established that, within the galactomannan series, the extent of synergistic interaction increases with decreasing content of galactose sidechains, prompting an early proposal (Dea et al., 1972) that interaction occurs by attachment of unsubstituted ('smooth') regions of the mannan backbone to the ordered structure of the co-synergist, with more heavily substituted ('hairy') regions acting as interconnecting sequences in the hydrated network.

One line of reasoning behind this proposal was that conformational rigidity in one of the interacting species would lower the entropic disadvantage of intermolecular association, and thus facilitate formation of heterotypic junctions (Flory, 1956). The possibility that plant polysaccharides known to interact with xanthan and gelling agars and carrageenans might, therefore, also bind to other conformationally rigid polysaccharide structures was explored in the present work.

Two types of ordered materials were studied: the anionic bacterial polysaccharides welan and rhamsan, which are being developed as new industrial polymers (Pettitt, 1986), and the 'egg-box' assemblies formed by alginate and pectin in the presence of calcium ions (Grant et al., 1973). The plant polysaccharides chosen for investigation were locust bean gum (LBG), a galactomannan of low galactose content, and konjac glucomannan, commonly known as konjac mannan (KM), both of which interact strongly with xanthan and algal polysaccharides in the agar/carrageenan family.

To distinguish between direct binding and thermodynamic incompatibility (i.e. between unlike chains 'sticking together' and 'pushing apart'), the mixed systems were characterized by measurements of viscosity in very dilute solution where exclusion effects should be negligible. Application of the same approach to binary combinations of LBG or KM with xanthan or de-acetylated xanthan has been reported elsewhere (Foster & Morris, 1994), and showed massive increases in viscosity on mixing dilute solutions of the individual polysaccharides.

A second objective of the present investigation was to explore the effect of ionic environment on the interaction of (1 → 4)-ß-glycans with carrageenans. The carrageenan backbone consists of a linear alternating sequence of (1 → 3)-linked ß-D-galactose and (1 → 4)-linked α-D-galactose. Adoption of the double helix structure involved in gel formation can occur only when the 4-linked residues are present as the 3,6-anhydride (Rees, 1972; Rees et al., 1982). Lambda carrageenan, in which the anhydride bridge is absent, is non-gelling. Commercial gelling carrageenans are hybrids (Parker et al., 1993) of (predominantly) two idealized repeating structures: kappa carrageenan, which has one sulphate group per disaccharide, and iota carrageenan, which has a sulphate group on every residue (i.e. two charges per disaccharide).

Gelation of kappa carrageenan is promoted specifically by K^+ (and larger Group I cations), which bind to the double helix structure (Rochas & Rinaudo, 1980; Piculell et al., 1993) and allow the helices to associate into larger assemblies. Dissociation of helix–helix aggregates occurs at higher temperature than initial formation of individual helices on cooling, with consequent thermal hysteresis between gelation and melting (Morris & Norton, 1983). Other cations, notably Me_4N^+, can induce helix formation without detectable hysteresis or gelation, by non-specific screening of electrostatic repulsion between the participating strands. The temperature of the disorder–order transition is also extremely sensitive to the nature of the counteranion, with iodide salts being particularly effective in promoting conformational ordering (Norton et al., 1984).

Iota carrageenan, by contrast, is unaffected by differences in anionic environment and shows no

evidence of specific site-binding of metal ions. However, since it has a much higher charge density than kappa, it is correspondingly more effective in inducing 'condensation' of counterions around the polymer chain or, particularly, around the double helix. The degree of condensation is also dependent on the charge of the counterions, and therefore follows the order: kappa + monovalent < kappa + divalent ≈ iota + monovalent < iota + divalent.

The polymer–salt combinations used in the present work were: iota + $CaCl_2$ (to give strong atmospheric binding); iota + KCl (to give weaker binding with, consequently, greater electrostatic repulsion between individual helices); kappa + KCl (to explore the effect of helix–helix aggregation induced by specific site-binding of counterions) and kappa + Me_4NI (at a concentration sufficient to promote full conversion to the ordered form). In all cases the carrageenan component was first ion-exchanged to the appropriate salt form, so that only one type of counterion was present in the ionic environment of the polymer chains.

MATERIALS AND METHODS

A sample of KM from Senn Chemicals AG was kindly supplied by Dr V.J. Morris (Institute of Food Research, Norwich, UK) and was from the same batch as the material used in the X-ray diffraction studies of Cairns *et al.* (1988). The powder was allowed to swell overnight in water (at ~2% w/w) and was dissolved by autoclaving for 20 min at 120°C. The solution was clarified by centrifugation and dialysed for 2 days against four changes of water, and the final concentration was determined by freeze-drying a weighed aliquot. A stock solution of LBG (Meypro fleur M-175 from Meyhall) was prepared in the same way, but with initial dispersion in water in place of the pre-swelling step. All other polysaccharides were dissolved by mechanical stirring at ambient temperature, using distilled deionized water throughout.

Welan (batch number 88074A), rhamsan (batch number 92056A), alginate (Manugel DMB; high guluronate) and xanthan (Keltrol T) were gifts from the Kelco Division of Merck and Co. Inc. Pectin (low-methoxy), iota carrageenan (X6955) and kappa carrageenan (X6960) were from the Copenhagen Pectin Division of Hercules. The iota carrageenan contained 5–10% of kappa sequences, as determined by the ^{1}H NMR method of Welti (1977), with a similar level of iota content in the kappa carrageenan sample.

Potassium and tetramethylammonium salt forms of kappa carrageenan were prepared by cation exchange on Amberlite IR-120 from BDH. The resin was first converted to the H^+ form by elution with HCl, and then to the K^+ or Me_4N^+ form using the appropriate chloride salts. Excess salt was removed by elution with

water until the washings no longer gave a precipitate with silver nitrate (i.e. chloride free). Ion exchange of the carrageenan sample was carried out at high temperature to maintain the polymer in the disordered coil form. The solution was then dialysed against water, and the final concentration was again determined by freeze-drying a weighed aliquot. Potassium and calcium salt forms of iota carrageenan were prepared in the same way. Tetramethylammonium chloride and iodide were from Aldrich; all other reagents were AnalR grade from BDH.

Stock solutions for studies of viscous interaction were dialysed together against three changes of the appropriate salt solution, to eliminate changes in ionic environment on mixing. The samples were weighed before and after dialysis, and allowance was made for any changes in volume when calculating polymer concentrations. The final dialysate was used for all subsequent dilutions. Mixtures were prepared by accurate weighing at ambient temperature.

Viscosity measurements were made at 20°C on a Contraves Low Shear 30 viscometer, using concentric cylinder geometry with inner and outer radii of 5.5 and 6.0 mm, respectively. The instrument was interfaced with an external drive device to increase and decrease the shear rate linearly between 0 and $100 \, s^{-1}$ over a total period of 2 min. Flow curves of shear stress vs shear rate were plotted directly on an *X–Y* recorder. Low-amplitude oscillatory measurements of storage modulus (G'), loss modulus (G'') and complex dynamic viscosity (η^*) were made using cone-and-plate geometry (cone angle 0.05 rad; diameter 5 cm) on a sensitive prototype rheometer designed and constructed by Dr R.K. Richardson (Silsoe College). In both cases temperature was controlled by a circulating water bath and measured by a thermocouple in direct contact with the stationary element.

RESULTS

The method used to screen for binding interactions in dilute solution is illustrated in Fig. 1 for mixtures of LBG and xanthan (which, although individually non-gelling, form synergistic gels at higher concentrations). Solutions of the two polysaccharides were dialysed together against 10 mM KCl, and were diluted with the dialysate until both had $\eta_{sp} \approx 1$ (i.e. viscosity about twice that of the dialysate). Flow curves of shear stress (σ) vs shear rate ($\dot{\gamma}$) were then recorded (at 20 °C) as $\dot{\gamma}$ was increased and decreased over the range $0–100 \, s^{-1}$. Similar flow curves were recorded for various mixtures of the individual stock solutions.

In the illustrative example shown in Fig. 1, the relative volumes used were chosen to give a xanthan:LBG ratio of 7:3. The resistance (shear stress) of the mixed solution is substantially higher than that of
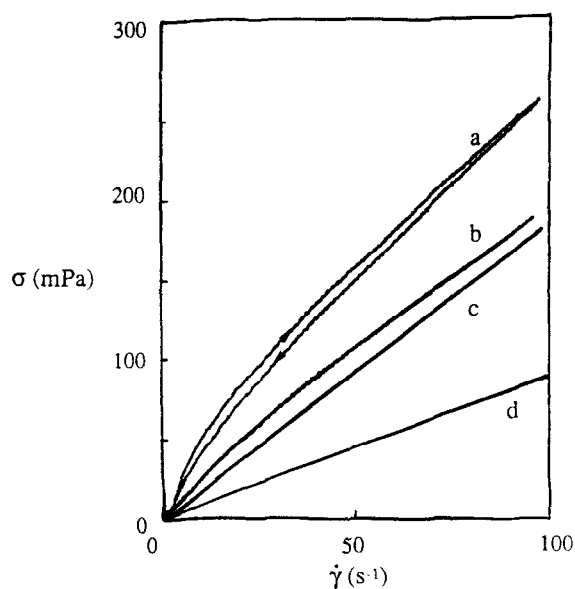
Fig. 1. Flow curves (20°C) of shear stress (σ) vs shear rate ($\dot{\gamma}$) for stock solutions ($\eta_{sp} \approx 1$) of xanthan (0.008% w/w) and LBG (0.08% w/w) dialysed together against 10 mM KCl, and for an illustrative mixture with a xanthan:LBG ratio of 7:3. (a) mixed solution (arrows indicate increasing and decreasing $\dot{\gamma}$); (b) xanthan alone; (c) LBG alone; (d) dialysate; traces for (b), (c) and (d) gave close superposition on increasing and decreasing $\dot{\gamma}$.

Fig. 2. Specific viscosity (measured at 20°C and 50 s⁻¹ as $\dot{\gamma}$ was increased) for dilute solutions of LBG in combination with (a) lambda carrageenan in 100 mM KCl; and (b) xanthan in 10 mM KCl; f denotes the LBG content expressed as a fraction of the total polymer concentration; the concentrations (% w/w) of the individual polymer solutions were: (a) 0.063 LBG, 0.304 carrageenan; and (b) 0.080 LBG, 0.008 xanthan (as in Fig. 1).

the constituent solutions, indicating an increase in overall hydrodynamic volume by intermolecular association. In contrast to the individual stock solutions, the mixed system also shows significant thixotropy (i.e. with values of σ recorded on deceleration falling below those obtained as $\dot{\gamma}$ was increased), which is again indicative of intermolecular association.

The dependence of the properties of the mixed solutions on the relative proportions of xanthan and LBG is shown in Fig. 2b, using viscosities ($\sigma/\dot{\gamma}$) calculated from shear stress in the centre of the shear rate range used (i.e. at 50 s⁻¹) as $\dot{\gamma}$ was increased. As has been frequently reported from studies at higher polymer concentration, the measured viscosities pass through a maximum when the two polymers are present in roughly equal amounts. The distinguishing feature of the present approach is that the polymer concentrations required to give $\eta_{sp} \approx 10$ correspond to a very low degree of space-occupancy ($\eta_{sp} = 1$ at the onset of entanglement; Morris *et al.*,) so that exclusion effects should be vanishingly small.

The absence of any significant contribution to overall viscosity from non-specific thermodynamic incompatibilities between unlike chains is confirmed directly by comparative studies of binary mixtures that show no evidence of synergistic interaction at higher concentrations. For example, the observed viscosities for LBG in combination with lambda carrageenan (which is conformationally disordered) remain close to
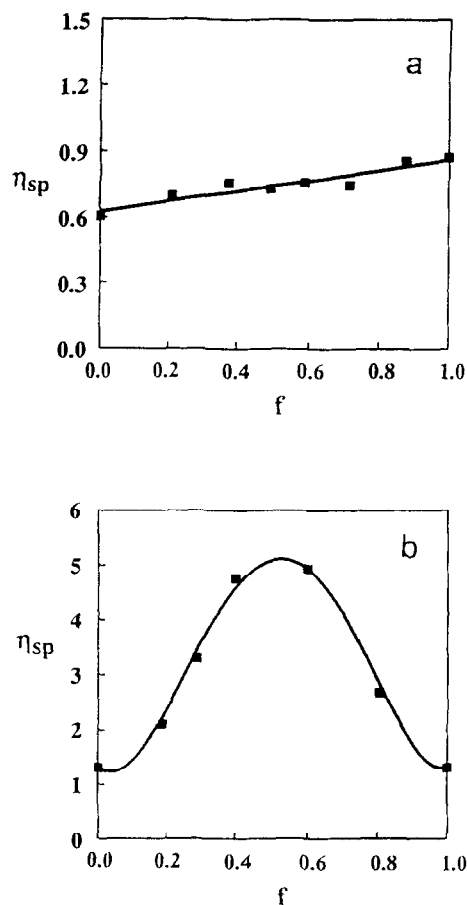
those of the individual stock solutions at all mixing ratios (Fig. 2a).

As mentioned previously, a unifying feature of the polysaccharides that are known to form synergistic gels with (1 → 4)-β-glycans such as LBG and KM is that they are all capable of adopting ordered structures. The possibility of other ordered polysaccharides (welan; rhamsan; calcium polyguluronate; calcium polygalacturonate) forming heterotypic junctions with LBG or KM was therefore explored.

Solutions of welan and rhamsan have 'weak gel' properties similar to those of xanthan, which is indicative of conformational order, but unlike xanthan they show no evidence of a conformational transition between 0 and 100°C, prompting the suggestion (Crescenzi *et al.*, 1987) that they occur as disordered coils. However, both give featureless high-resolution NMR spectra and show little change in intrinsic viscosity on varying ionic strength (Robinson *et al.*, 1991), arguing strongly for rigid chain geometry.

Table 1. Polysaccharide combinations showing no interaction in dilute solution (20°C)

Glycan	c (% w/w)	Other component	c (% w/w)	Solvent
KM	0.163	Welan	0.025	10 mM NaCl
KM	0.163	Rhamsan	0.006	10 mM NaCl
LBG	0.163	Rhamsan	0.006	10 mM NaCl
LBG	0.063	Pectin + Ca^{2+}	0.036	10 mM NaCl
LBG	0.063	Alginate + Ca^{2+}	0.052	10 mM NaCl
LBG	0.063	i-carrageenan	0.063	100 mM KCl
LBG	0.063	κ-carrageenan*	0.010	Water
LBG	0.063	λ-carrageenan*	0.304	100 mM KCl

*Disordered.

Conformational order in welan has recently been demonstrated directly (Hember *et al.*, 1994) by using dimethyl sulphoxide (DMSO) to destabilize the ordered structure and monitoring the order–disorder transition induced by changes in temperature and solvent composition in DMSO–water mixtures. An order–disorder transition has also been observed for rhamsan, by using deacetylation under mild alkaline conditions to reduce the thermal stability of the ordered structure (E.R. Morris and M.W.N. Hember, unpublished).

The poly-L-guluronate sequences of alginate and poly-D-galacturonate sequences of pectin both form dimeric 'egg-box' junctions (Morris *et al.*, 1978, 1882) incorporating calcium ions at half the stoichiometric equivalent of the polysaccharide carboxyl groups (since only the inner faces of the chains in the two-fold ordered conformation are involved in site-binding of Ca^{2+}). In preparing alginate and pectin solutions for the viscometric studies, 'egg-box' formation was induced by including calcium chloride in the final dialysate, at a concentration equivalent to half the full stoichiometric requirement of the polymer chains.

The results from these experiments were entirely negative. Mixed solutions of welan, rhamsan or the ordered 'egg-box' assemblies with LBG or KM behaved in the same way as the lambda carrageenan/LBG mixtures shown in Fig. 2a, with the measured viscosities remaining close to those of the individual stock solutions at all mixing ratios. The combinations studied, the ionic conditions used, and the polymer concentrations required to give $\eta_{sp} \approx 1$ are listed in Table 1.

The table also includes three other systems that gave negative results: LBG in combination with (a) disordered lambda carrageenan (Fig. 2a); (b) disordered kappa carrageenan (0.01% w/w in water); and (c) the ordered conformation of iota carrageenan in 100 mM KCl (Fig. 3a). In the presence of calcium ions (90 mM CaCl₂), however, mixed solutions of iota carrageenan and LBG showed a significant reduction in viscosity (Fig. 3b). A similar decrease was also observed (Fig. 4) for LBG in combination with the ordered conformation of Me_4N^+ kappa carrageenan (in 150 mM Me₄NI).

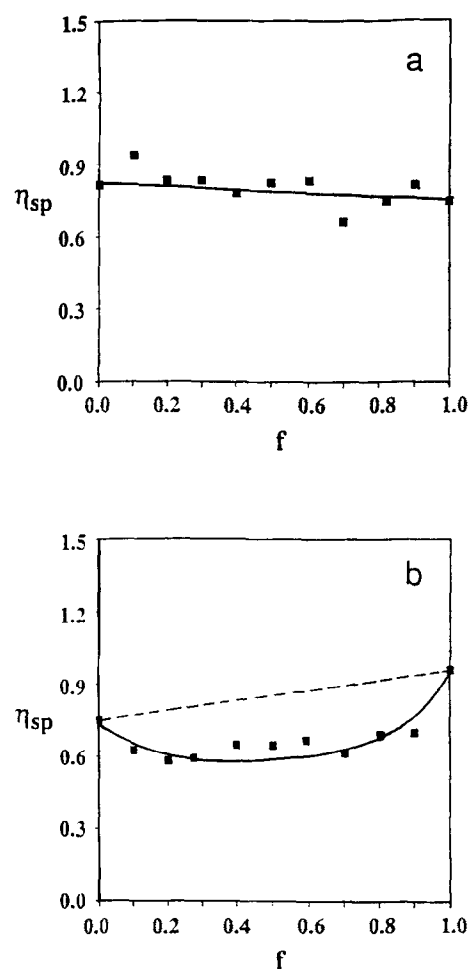An attempt was made to carry out an analogous

Fig. 3. Specific viscosity (measured at 20°C and 50 s⁻¹ as $\dot{\gamma}$ was increased) for dilute solutions of iota carrageenan and LBG in (a) 100 mM KCl; and (b) 90 mM CaCl₂; *f* denotes the LBG content expressed as a fraction of the total polymer concentration; the concentrations (% w/w) of the individual polymer solutions were: (a) 0.063 LBG, 0.063 carrageenan; and (b) 0.069 LBG, 0.047 carrageenan.

viscometric study with kappa carrageenan in the K^+ salt form. Two solutions of LBG (equilibrated against 100 mM KCl) were prepared to specific viscosities of ~0.9 and ~2.4, the polymer concentrations used being 0.075 and 0.157% w/w, respectively. The concentrations of carrageenan required to give approximately the same
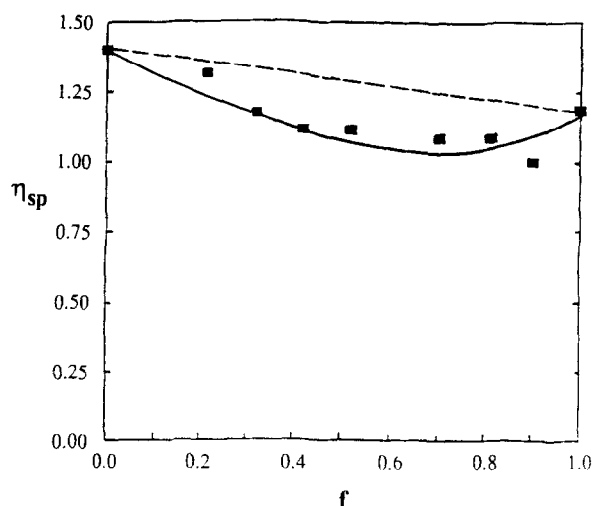
Fig. 4. Variation in specific viscosity (20°C) on mixing solutions of Me_4N^+ kappa carrageenan (0.085% w/w) and LBG (0.078% w/w) in various proportions. Both solutions were equilibrated against 150 mM Me_4NI; f denotes the fraction of LBG solution in each mixture.

viscosities towards the centre of the shear rate range used (i.e. at $\sim 50\,s^{-1}$) were ~ 0.010 and $\sim 0.025\%$ w/w. However, even at these extremely low concentrations, the flow curves recorded for K^+ kappa carrageenan alone showed appreciable shear thinning and thixotropy (Fig. 5). On mixing with LBG, the response became extremely erratic, with sharp peaks and troughs in shear

stress, indicating the presence of a dynamic network breaking under the imposed deformation and then immediately re-forming before again being broken.

The presence of a network structure, both in the mixed solutions and in the carrageenan starting solution, was confirmed by small-deformation oscillatory measurements over three decades of frequency (ω). Figure 6 shows the mechanical spectra obtained for the carrageenan solution from Fig. 5b, alone, and in two representative mixtures with LBG. In all cases the spectra have obvious gel-like character ($G' > > G''$ linear variation of $\log \eta^*$ with $\log \omega$; little frequency dependence of G'). The storage moduli for the mixed solutions were greatest when the two polymers were present in approximately equal amounts ($\sim 0.022\%$ w/w of both; Fig. 6c), giving $G' \sim 8\,Pa$ in comparison with $G' \approx 1\,Pa$ for the carrageenan starting solution (Fig. 6a). G' values for the starting solution of LBG were too low to be measured, even on the very sensitive instrument used ($< 0.1\,Pa$).

DISCUSSION

As expected, the disordered carrageenans studied (K^+ kappa in water; lambda in 100 mM KCl) showed no evidence of interaction with LBG. Adoption of double helix geometry, however, does not appear to be sufficient to promote association since, as anticipated
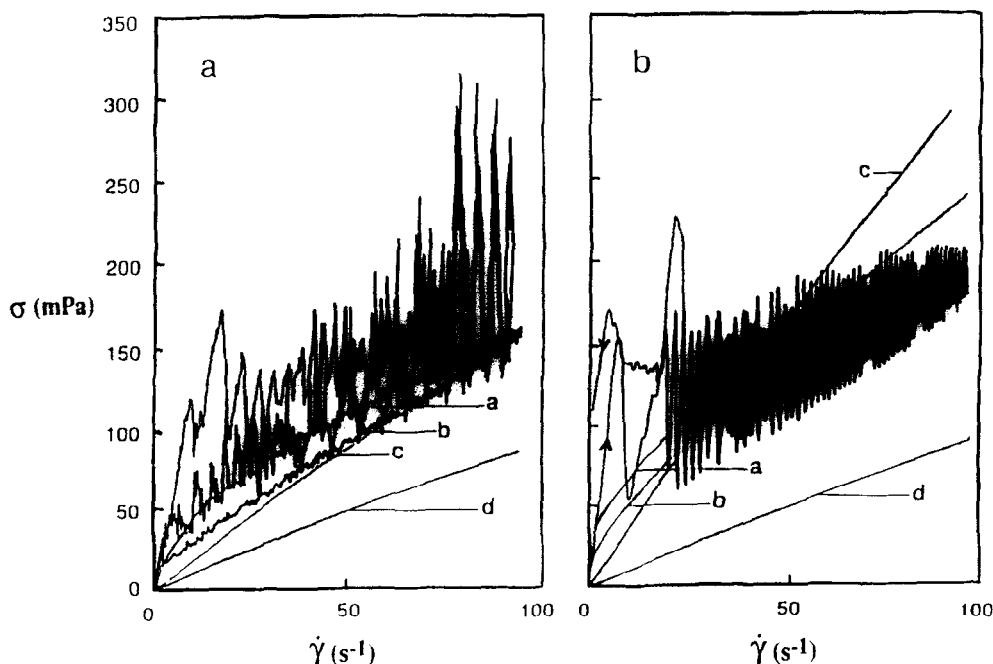

Fig. 5. Flow curves (20°C) for solutions of K^+ kappa carrageenan and LBG, both equilibrated against 100 mM KCl, and for mixtures incorporating 70% carrageenan solution and 30% LBG solution. The curves labelled (a) and (b) were recorded for the carrageenan solution on, respectively, increasing and decreasing the shear rate; curves (c) and (d) are for, respectively, the LBG solution and the KCl dialysate (with close superposition on acceleration and deceleration); the erratic traces were recorded for the mixed solutions as shear rate was increased and decreased. The concentrations (% w/w) of the individual solutions were: (a) 0.010 carrageenan, 0.075 LBG; and (b) 0.025 carrageenan; 0.157 LBG.
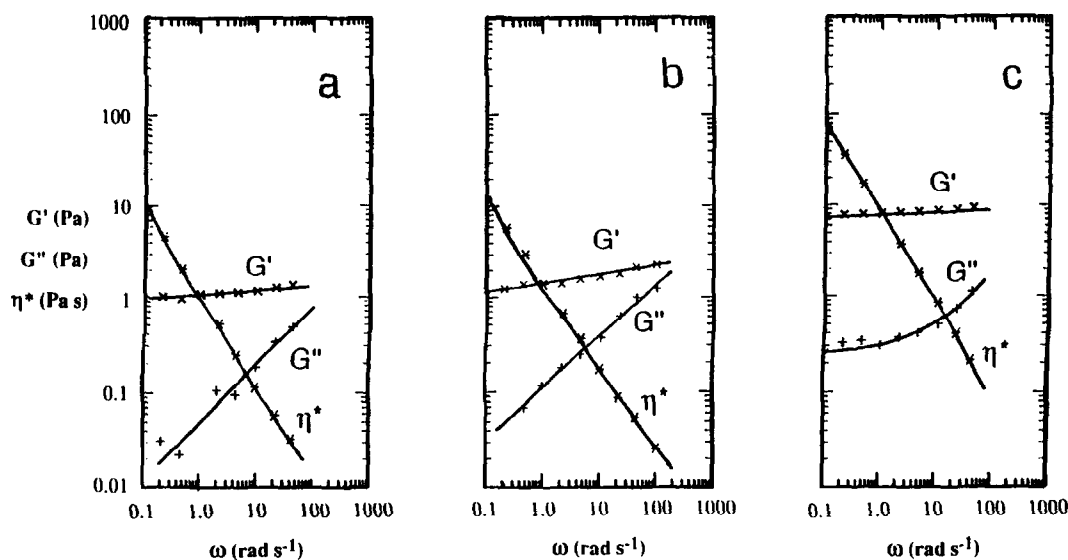
Fig. 6. Mechanical spectra (20°C; 2% strain) for mixtures of the solutions from Fig. 5b: 0.025% K$^+$ carrageenan and 0.157% LBG, both equilibrated against 100 mM KCl. The fraction of LBG solution present was: (a) 0 (i.e. carrageenan alone); (b) 50% and (c) 13.8% (the ratio giving the maximum enhancement of G').

from previous studies (e.g. Dea *et al.*, 1972), no interaction was observed with the ordered form of iota carrageenan in potassium chloride solution (Fig. 3a). Welan, rhamsan and the ordered 'egg-box' structures of alginate and pectin also showed no evidence of interaction (Table 1), again demonstrating that conformational rigidity is not, in itself, sufficient to allow other polysaccharides to form heterotypic junctions with galactomannan or glucomannan chains.

However, although iota carrageenan showed no indication of interaction with LBG in an environment of monovalent cations (100 mM KCl), a substantial reduction in viscosity was observed in calcium chloride solution (Fig. 3b), indicating that, under these conditions, the galactomannan can bind to the polymer and cause a contraction in the hydrodynamic volume of microgel 'domains'. A possible explanation for the difference in behaviour under these different ionic conditions is that calcium ions, because of their higher charge, promote some limited aggregation of iota carrageenan helices, and that it is the aggregates, rather than the individual helices, which form associations with the galactomannan chains.

A similar effect was observed (Fig. 4) for the Me$_4$N$^+$ salt form of kappa carrageenan in the presence of a high concentration of Me$_4$NI (150 mM), and can be interpreted in the same way. Tetramethylammonium ions are highly unlikely to form coordination complexes with polyanions (i.e. to participate in site-binding) and are therefore often used in research to explore conformational ordering in the absence of cation-mediated aggregation (e.g. Morris *et al.*, 1980; Crescenzi *et al.*, 1987). However, at sufficiently high concentration they can induce helix–helix aggregation by non-specific screening of

electrostatic repulsion. In particular, gellan gum, which has a somewhat lower charge density than kappa carrageenan and forms gels by aggregation of double helices, will give a cohesive network with Me$_4$N$^+$ as the sole counterion present (Crescenzi *et al.*, 1987), although the cation concentration needed is extremely high ($\sim$700 mM, in comparison with $\sim$70 mM for Na$^+$ and $\sim$4 mM for Ca^{2+}). Thus some slight aggregation of kappa carrageenan helices under the salt conditions used in the present work (150 mM Me$_4$NI) seems entirely feasible.

Finally, the results shown in Figs 5 and 6 clearly demonstrate the formation of a long-range network structure by mixtures of LBG and kappa carrageenan in the presence of high concentrations of K$^+$ (100 mM KCl) and, because of the very low polymer concentrations used, argue strongly for direct binding rather than any form of exclusion mechanism. As discussed previously, potassium ions promote extensive aggregation of kappa carrageenan by site-binding to the double helix. It therefore seems reasonable to conclude that helix–helix aggregation is centrally involved in the very strong 'synergistic' interactions observed, the most likely interpretation being that association occurs by attachment of (1 → 4)-ß-glycan chains to the surface of the aggregated assemblies.

ACKNOWLEDGEMENTS

REFERENCES

Cairns, P., Miles, M.J. & Morris, V.J. (1988). *Carbohydr. Polym.*, **8**, 99–104.

Crescenzi, V., Dentini, M. & Dea, I.C.M. (1987). *Carbohydr. Res.*, **160**, 283–302.

Dea, I.C.M., McKinnon, A.A. & Rees, D.A. (1972). *J. Mol. Biol.*, **68**, 153–172.

Dea, I.C.M. & Morrison, A. (1975). *Adv. Carbohydr. Chem. Biochem.*, **31**, 241–312.

Dea, I.C.M. & Rees, D.A. (1987). *Carbohydr. Polym.*, **7**, 183–224.

Flory, P.J. (1956). *Proc. R. Soc. Ser. A*, **234**, 73–89.

Foster, T.J. & Morris, E.R. (1994). In *Gums and Stabilisers for the Food Industry 7*, eds G.O. Phillips, P.A. Williams & D.J. Wedlock, IRL Press, Oxford, pp. 281–289.

Grant, G.T., Morris, E.R., Rees, D.A., Smith, P.J.C. & Thom, D. (1973). *FEBS Lett.*, **32**, 195–198.

Hember, M.W.N., Richardson, R.K. & Morris, E.R. (1994). *Carbohydr. Res.*, **252**, 209–221.

Kasapis, S. & Morris, E.R. (1993). In *Food Hydrocolloids: Structures, Properties and Functions*, eds K. Nishinari & E. Doi. Plenum Press, New York, pp. 97–103.

Morris, E.R., Cutler, A.N., Ross-Murphy, S.B., Rees, D.A. & Price, J. (1981). *Carbohydr. Polym.*, **1**, 5–21.

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

Morris, E.R., Powell, D.A., Gidley, M.J. & Rees, D.A. (1982). *J. Mol. Biol.*, **155**, 507–516.

Morris, E.R., Rees, D.A. & Robinson, G. (1980). *J. Mol. Biol.*, **138**, 349–362.

Morris, E.R., Rees, D.A., Thom, D. & Boyd, J. (1978). *Carbohydr. Res.*, **66**, 145–154.

Norton, I.T., Morris, E.R. & Rees, D.A. (1984). *Carbohydr. Res.*, **134**, 89–101.

Parker, A., Brigand, G., Miniou, C., Trespoey, A. & Vallée, P. (1993). *Carbohydr. Polym.*, **20**, 253–262.

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

Piculell, L., Nilsson, S., Viebke, C. & Zhang, W. (1993). In *Food Hydrocolloids: Structures, Properties and Functions*, eds K. Nishinari & E. Doi. Plenum Press, New York, pp. 35–44.

Rees, D.A. (1972). *Biochem. J.*, **126**, 257–273.

Rees, D.A., Morris, E.R., Thom, D. & Madden, J.K. (1982). In *The Polysaccharides*, ed. G.O. Aspinall. Academic Press, New York, Vol. 1, pp. 195–290.

Robinson, G., Manning, C.E. & Morris, E.R. (1991). In *Food Polymers Gels and Colloids*, ed. E. Dickinson. Royal Society of Chemistry, Cambridge, pp. 22–33.

Rochas, C. & Rinaudo, M. (1980). *Biopolymers*, **19**, 1675–1687.

Thom, D., Dea, I.C.M., Morris, E.R. & Powell, D.A. (1982). *Prog. Food Nutr. Sci.*, **6**, 97–108.

Toft, K. (1982). *Prog. Food Nutr. Sci.*, **6**, 89–96.

Toft, K., Grasdalen, H. & Smidsrød, O. (1986). *ACS Symp. Ser.*, **310**, 117–132.

Tolstoguzov, V.B. (1986). In *Functional Properties of Food Macromolecules*, eds J.R. Mitchell & D.A. Ledward. Elsevier, London, pp. 385–415.

Tolstoguzov, V.B. (1988). *Food Hydrocoll.*, **2**, 339–370.

Tolstoguzov, V.B. (1991). *Food Hydrocoll.*, **4**, 429–468.

Welti, D. (1977). *J. Chem. Res. (M)*, 3566–3587.