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Preparation, authentication, rheology and conformation of theta carrageenan

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ABSTRACT

Lambda carrageenan was extracted from hand-sorted tetrasporophytes of *Gigartina skottsbergii*. Theta carrageenan was prepared from it by treatment with alkaline borohydride. Infrared spectroscopy showed complete, or essentially complete, conversion of 4-linked residues to the 3,6-anhydride ring form, and no detectable contamination with gelling carrageenans (iota or kappa). Optical rotation and differential scanning calorimetry (DSC) showed no evidence of conformational transitions on cooling and heating. Solutions of theta carrageenan at 2.0 wt.% in water and in 0.1 M KCl were Newtonian at 20 °C, and substantially less viscous than a 1.0 wt.% solution of the lambda carrageenan precursor. Close Cox–Merz superposition was observed between steady-shear (rotational) viscosity (η) and complex dynamic viscosity (η^*) at equivalent values of shear rate ($\dot{\gamma}$/s^{-1}) and frequency (ω/rad s^{-1}), and the variation of log G'' with log ω was linear, with a slope of 1.0, strongly indicating a solution of disordered coils. The absence of double-helix formation, and associated gelation, is explained by sulfation at O(2) of 3-linked residues. A previous report of gel formation by theta carrageenan is attributed to the presence of some gelling carrageenan in the lambda sample from which it was prepared.

1. Introduction

The carrageenans are a family of sulfated, linear polysaccharides that occur (Therkelsen, 1993) in the cell wall and intercellular matrix of numerous species of red seaweeds (Rhodophyta). Their primary structures are based on an alternating disaccharide repeating sequence of β-D-galactose linked at position 3, and α-D-galactose linked at position 4. Carrageenans have extensive industrial applications as thickeners, stabilisers and gelling agents in food and related products (such as cosmetics, toiletries and toothpaste).

Carrageenan gels form on cooling and melt on heating. The primary step in the gelation process is conversion of disordered coils to coaxial double helices (McKinnon, Rees, & Williamson, 1969; Rees, 1970). Further association can occur by helix–helix aggregation (Morris, Rees, & Robinson, 1980b; Piculell, 1995), which is promoted by metal cations (typically K^+) and gives rise to thermal hysteresis between the sol–gel and gel–sol transitions. Sufficiently high concentrations of K^+ can cause excessive aggregation, resulting in precipitation. Fractional precipitation with potassium chloride was used as a basis for classification of carrageenans in early studies (e.g. Pernas, Smidsrød, Larsen, & Haug, 1967; Smith & Cook, 1953). Carrageenans are now, however, normally classified by their chemical structure, whose elucidation came mainly from an extensive series of investigations by Rees and his co-workers in the 1960s. The understanding arising from these investigations, and related studies, is summarised by Stanley (1990) and outlined below.

The principal gelling carrageenans are iota and kappa. In both of these, the 4-linked residues occur predominantly as 3,6-anhydrides. Closure of the anhydride bridge requires conversion of the sugar ring from the normal $^4C_1$ chair form (with C(6) equatorial) to the $^1C_4$ form, which in turn converts the linkages at C(1) and C(4) from axial to equatorial. Only the diequatorially-linked arrangement is compatible with formation of the double-helix structure. Sequences in which the anhydride bridge is absent cause a "kink" in chain geometry and terminate helix propagation. Insertion of the anhydride bridge *in vivo* occurs (Lawson & Rees, 1970) by the action of an enzyme termed "dekinkase". Iota carrageenan is biosynthesised as a soluble precursor, nu carrageenan, in which the 4-linked residues are in the normal $^4C_1$ chair form (with axial linkages at C(1) and C(4)). Progressive conversion to the gelling form, which confers structure to the plant tissue, then occurs by the action of the dekinkase enzyme. The corresponding soluble precursor of kappa carrageenan is mu, which is converted to the gelling form in the same way.

Both nu and mu carrageenan have a sulfate group on O(6) of the 4-linked residue (Fig. 1). Insertion of the anhydride bridge therefore involves formal elimination of a molecule of sulfuric acid,

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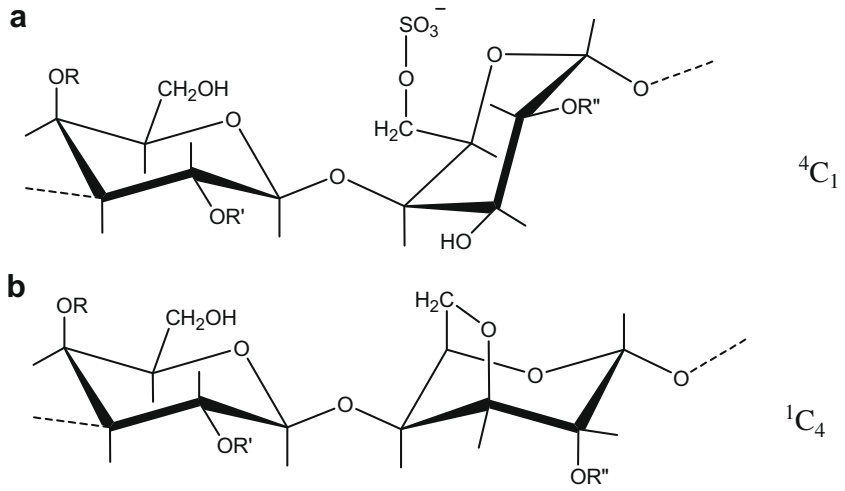

Fig. 1. Idealised disaccharide repeat units of (a) mu, nu and lambda carrageenan, (b) kappa, iota and theta carrageenan. Their pattern of sulfation is as follows. mu and kappa: R = SO_3^-, R′ = H, R″ = H; nu and iota: R = SO_3^-, R′ = H, R″ = SO_3^-; lambda and theta: R = H, R′ = SO_3^-, R″ = SO_3^-.

and can be induced by treatment with alkali (Smidsrød, Larsen, Pernas, & Haug, 1967; Stanley, 1963). "Alkali modification" is now used as a standard industrial procedure to enhance structural regularity (and gel strength) of iota and kappa carrageenan (Stanley, 1990; Therkelsen, 1993), by reducing the content of 4-linked residues in which the anhydride bridge is absent.

Although naturally-occurring carrageenans show a spectrum of heterogeneity (Pernas et al., 1967) corresponding to the extent of action of the dekinkase enzyme, their conformations and functional properties can be rationalised by the idealised disaccharide repeating sequences shown in Fig. 1. In these idealised structures, there is a sulfate group on O(4) of the 3-linked residue in both iota and kappa carrageenan, and their respective precursors (nu and mu). In iota (and nu), however, there is an additional sulfate group on O(2) of the 4-linked residue, giving two sulfates per disaccharide repeat unit in iota, but only one in kappa. This difference in sulfation causes some modification of the geometry of the double-helix, as characterised by X-ray diffraction of oriented fibres in the solid state.

In iota carrageenan, the two strands of the helix run parallel to one another. Each has 3-fold symmetry, with a repeat distance of 2.6 nm along the helix axis. The strands, however, are exactly staggered (i.e. one is displaced relative to the other by a rotation of 180° and translation of half the pitch of the individual helices), giving a repeat distance of 1.3 nm for the coaxial duplex (Anderson, Campbell, Harding, Rees, & Samuel, 1969; Arnott, Scott, Rees, & McNab, 1974). The double-helix is stabilised by intermolecular hydrogen bonds between O(2) and O(6) of the 3-linked residues of the two strands (the only unsubstituted hydroxyl groups in the primary structure).

Although the X-ray fibre diffraction patterns obtained for kappa carrageenan are of poorer quality than those of iota, they suggest similar double-helix geometry (Millane, Chandrasekaran, Arnott, & Dea, 1988). The perfect staggering of the two strands, however, is lost, and the best fit to the diffraction data indicates only one O(2)–O(6) hydrogen bond per disaccharide unit of each strand, in comparison with two for iota.

A third carrageenan of commercial significance is lambda. The 4-linked residues in lambda carrageenan exist entirely in the normal $^4C_1$ ring form, with no 3,6-anhydride bridge. The polymer is therefore analogous to the nu and mu precursors of, respectively, iota and kappa carrageenan, and, like these precursors, is non-gelling. The similarity of lambda carrageenan to nu and mu extends to the presence of a sulfate group at C(6) of the 4-linked residue and a hydroxyl group at C(3). Insertion of the anhydride bridge can again, therefore, be induced by alkali modification (Ciancia, Noseda, Matulewicz, & Cerezo, 1993; Morris, Rees, Welsh, Dunfield, & Whittington, 1978; Rees, 1961, 1963), giving a product termed theta carrageenan. Occurrence of theta carrageenan in nature was reported only comparatively recently (Falshaw, Bixler, & Johndro, 2001), when it was detected in trace amounts in two Chilean seaweeds, *Sarcothalia crisptata* and *Gigartina skottsbergii*. Subsequently, however, it was found (Falshaw, Furneaux, & Stevenson, 2005) as the most abundant polysaccharide in *Callophyllis hombroniana*, a species indigenous to New Zealand.

Like nu and iota carrageenan, but unlike mu and kappa, lambda carrageenan (and hence theta carrageenan derived from it) has a sulfate group on O(2) of the 4-linked residue in the disaccharide repeating sequence (Fig. 1). In contrast to the sequences of these other carrageenans (nu, mu, iota and kappa), however, the 3-linked residue has no sulfate group at O(4), but instead is sulfated at O(2).

Sulfation of theta carrageenan at O(2) of 3-linked residues (as in the lambda carrageenan from which it is derived) would be expected to prevent double-helix formation (and hence gelation), first by eliminating the stabilising effect of the O(2)–O(6) hydrogen bond, and, more significantly, by introducing a bulky sulfate in a position which, in the double-helix structure of iota and kappa carrageenan, is occupied by a hydrogen atom buried within the core of the helix. Gelation of theta carrageenan (on the basis of visual observations) has, however, been reported by Thành et al. (2002).

In the present work we have prepared a sample of pure theta carrageenan by alkali modification of its lambda carrageenan precursor, verified its structural authenticity by infrared spectroscopy, and characterised its conformational and rheological properties.

2. Materials and methods

2.1. Reagents

The sodium borohydride ($NaBH_4$) used in the preparation of theta carrageenan was from Aldrich, and the sodium hydroxide from Merck. The potassium chloride used in the studies of conformation and rheology was AnalaR grade from BDH. Distilled deionised water was used throughout.

2.2. Preparation of carrageenans

Lambda carrageenan was obtained by hand-sorting tetrasporic plants of *G. skottsbergii* into gametophytes, which yield carra-

geenan with disaccharide units of the kappa/iota type, and tetrasporophytes, which contain only lambda (McCandless, Craigie, & Walter, 1973). Separation was based on visual inspection: gametophytes are light red; tetrasporophytes have a much darker red colour, verging on black. Also, mature gametophytes have papillae which do not occur in tetrasporophytes. Lambda carrageenan was extracted at pH 8 from the hand-sorted tetrasporophytes; the extract was filtered hot, and the carrageenan was precipitated in 80% v/v isopropanol, freeze-dried and milled.

Investigation of the resulting material by ^{1}H and ^{13}C NMR (Dyrby et al., 2004; Guibet, Kervarec, Génicot, Chevolot, & Helbert, 2006) has shown that the combined content of contaminating kappa and iota sequences is less than 3%, and that ~92% of the disaccharide units in the dominant lambda component have the idealised (tri-sulfated) structure shown in Fig. 1b. However, the remaining disaccharide units (~8%), distributed randomly along the polymer chains, have an extra sulfate group, at O(4) of the 3-linked residue. This slight "over-sulfation" of lambda carrageenan from *G. skottsbergii* contrasts with the "under-sulfation" observed (Anderson, Dolan, Lawson, Penman, & Rees, 1968a) for lambda carrageenan from *Chondrus crispus*, where the sulfate group at O(2) of the 3-linked residue is present in only ~70% of the disaccharide units.

Theta carrageenan was prepared by a procedure broadly similar to that used by Morris et al. (1978). Lambda carrageenan (6 g) was dissolved in water (400 mL) at room temperature, sodium borohydride (3 g) was added, and the solution was held for 24 h with gentle stirring. Alkali (200 mL of 3 M NaOH) was then added, together with additional sodium borohydride (1.5 g), and the solution was stirred gently for 28 h at 80 °C, cooled, filtered, and dialysed overnight against water, using a membrane with molecular-weight cut-off 12–14 kDa. The resulting theta carrageenan was then freeze-dried, milled, and sieved to DIN 24. The purpose (Morris et al., 1978; Rees, 1961) of the sodium borohydride used in the above procedure is to convert the reducing (aldehyde) end-groups of the polymer chains to sugar alcohols, and thus minimise degradation by the "base peeling" reaction (Aspinall, 1970) which proceeds from the reducing end of polysaccharide molecules under alkaline conditions.

2.3. Infrared (IR) spectroscopy

Carrageenan powder was placed, under pressure, on a Diamond ATR crystal (DuraSamplIR II from SensIR Technologies) and IR spectra were measured on an MB-series 155 spectrophotometer from BOMEM, with 2 cm^{-1} resolution. Each spectrum is composed of an average of 32 scans in the spectral range 1400–600 cm^{-1}.

2.4. Monitoring for conformational transitions

Measurements of optical rotation were made at 365 nm (mercury emission line) on a Perkin Elmer 241 polarimeter, using a cell of pathlength 1 cm.

Differential scanning calorimetry (DSC) measurements were made on a Setaram DSC III microcalorimeter at a scan rate of 0.3 °C/min, with water as thermal reference. Sample and reference pans (typical loading ~850 mg) were balanced to within ±0.5 mg.

Solutions for both procedures were prepared by dispersing the polysaccharide in water by mechanical (overhead) stirring at ambient temperature and dissolving by continued stirring at ~80 °C. The solutions were then cooled, returned to their initial weight by addition of water to correct for evaporation, and clarified by centrifugation. For incorporation of potassium chloride, where used, solutions of carrageenan and KCl were prepared individually at concentrations above those required in the final samples; appropriate weights of the individual solutions were then mixed by magnetic stirring at ambient temperature.

2.5. Rheological measurements

Measurements of solution viscosity (η) were made at 20 °C, using concentric cylinder geometry (inner and outer radii 5.5 and 6.0 mm, respectively) on a Contraves Low Shear 30 viscometer. Low-amplitude oscillatory measurements of storage modulus (G'), loss modulus (G'') and complex dynamic viscosity ($\eta^* = (G'^2 + G''^2)^{1/2}/\omega$, where ω is frequency of oscillation in rad s^{-1}) were made using highly-truncated cone and plate geometry (5 cm diameter; 0.05 rad cone angle) on a sensitive prototype rheometer designed and constructed by Dr. R.K. Richardson, formerly of Cranfield University, UK. Samples for rheological characterisation were prepared by the procedure described above (Section 2.4).

3. Results

3.1. Infrared spectroscopy

The IR spectrum obtained for the sample of theta carrageenan prepared by the procedure described in Section 2.2 is shown in Fig. 2a. The largest peaks are in the spectral region from ~1000 to ~1100 cm^{-1}; these arise (Bayley, 1955; Orr, 1954) from C–O stretching and C–O–H bending modes, which are, of course, prevalent in any polysaccharide. The other dominant feature is an intense absorption at ~1230 cm^{-1}, which is characteristic of sulfated polysaccharides (Bayley, 1955; Neely, 1957; Rees, 1963) and can be attributed to S=O stretching. The peaks that are diagnostic (Chopin & Whalen, 1993; Matsuhiro, 1996) of differences in primary structures of carrageenans, however, are in the spectral region from ~750 to ~1000 cm^{-1}, which is shown in expanded form in Fig. 2b.

There is a pronounced peak at ~830 cm^{-1}, which is known (Anderson et al., 1968b) to arise from sulfate groups on O(2) of galactose, and would therefore be expected (Fig. 1) both for theta carrageenan and for the lambda carrageenan from which it is derived. Three features, however, indicate complete, or essentially complete, conversion of lambda to theta. The first is the absence of a peak at ~820 cm^{-1}, the position characteristic (Anderson et al., 1968b) of sulfate groups on O(6) of galactose, which are present (Fig. 1a) in lambda carrageenan, but not in theta (Fig. 1b). The second is a peak at 805 cm^{-1}; this is known (Anderson et al., 1968b) to arise from sulfate groups at O(2) of 3,6-anhydro galactose, which is present (Fig. 1b) in theta carrageenan, but not in lambda (Fig. 1a). The third is a sharp peak at ~930 cm^{-1}, which comes from the 3,6-anhydride bridge (Black, Blakemore, Colquhoun, & Dewar, 1965). The absence of a detectable peak at ~845 cm^{-1}, the position characteristic of sulfate groups on O(4) of galactose (Anderson et al., 1968b), indicates (Fig. 1) that there is no significant contamination with kappa or iota carrageenan, or their biosynthetic precursors (mu and nu). Taken together with previous authentication of the lambda carrageenan precursor by NMR (Dyrby et al., 2004; Guibet et al., 2006), our evidence from IR spectroscopy therefore indicates that there are no major differences between the primary structure of the theta carrageenan preparation studied and the idealised disaccharide repeating sequence shown in Fig. 1b.

3.2. Optical rotation

Formation and melting of carrageenan gels on cooling and heating are accompanied by large, sigmoidal changes in optical rotation (Bryce, Clark, Rees, & Reid, 1982; Morris, Rees, Norton, & Goodall, 1980a; Morris et al., 1980b; Rees, Morris, Thom, & Madden,

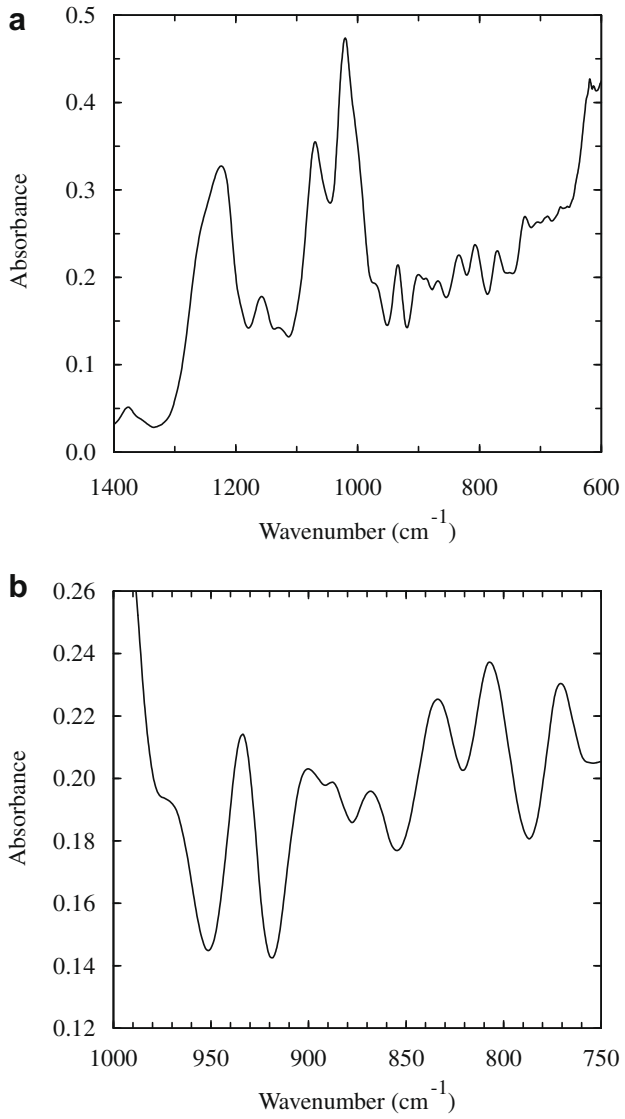

Fig. 2. (a) Infrared spectrum of theta carrageenan, (b) spectral region diagnostic of differences in primary structure of carrageenans, shown in expanded form.

1982). Closely similar changes were observed (McKinnon et al., 1969) for non-gelling segments of iota carrageenan (prepared by chain cleavage at "kinking" residues, and enhancement of structural regularity by alkaline borohydride), demonstrating that they arise from changes in conformation (coil–helix and helix–coil transitions), rather than from partial insolubilisation of the polysaccharide or any macroscopic properties of the gel network. Indeed, a convincing correlation has been demonstrated (Rees, Scott, & Williamson, 1970) between observed changes in optical rotation and values calculated by a semi-empirical analysis of the effect changes in the relative orientations of adjacent residues on the chiroptical properties of polysaccharide chains.

In the present work we have monitored the temperature-dependence of optical rotation for 2.0 wt.% theta carrageenan in 0.1 M KCl, conditions that would promote conformational ordering (and gelation) of iota or kappa carrageenan (Morris et al., 1980a, 1980b). The values recorded during cooling from ~95 °C are shown in Fig. 3. There is a small, essentially linear, increase in optical rotation as temperature is decreased, but no indication of the large, sigmoidal changes observed for the gelling carrageenans (iota and kappa).

3.3. Differential scanning calorimetry (DSC)

Dissociation of the carrageenan double-helix involves absorption of heat to break the non-covalent bonds between the two strands. This can be seen as an endothermic peak in DSC, with a corresponding exotherm from formation of double-helix structure on cooling (Morris et al., 1980a).

DSC heating and cooling traces recorded (at 0.3 °C/min) for 2.0 wt.% theta carrageenan in 0.1 M KCl (as in the optical rotation study reported above) are shown in Fig. 4. After the "start-up kick" of the calorimeter (initial thermal imbalance at beginning of the scan), the traces in both directions of temperature change are featureless, and show no evidence of thermal processes attributable to formation/dissociation of double helices.

3.4. Rheology

Visually, the sample of 2.0 wt.% theta carrageenan in 0.1 M KCl used in the studies of conformation reported in Sections 3.2 and 3.3 remained fluid, with little evident viscosity, between refrigerator temperature (~5 °C) and almost boiling (95 °C). To confirm and quantify this visual impression of very low-viscosity, objective measurements of the shear-rate dependence of viscosity (at 20 °C) for the same preparation are reported in Fig. 5, in comparison with corresponding traces for the same concentration of theta carrageenan in water, a lower concentration (1.0 wt.%) of the precursor lambda carrageenan in water, and water alone.

Lambda carrageenan (1.0 wt.% in water) shows detectable shear thinning (reduction in viscosity with increasing shear rate), as is commonly observed (Ross-Murphy, 1984) for solutions of entangled polymer coils. Theta carrageenan in water, at the higher concentration of 2.0 wt.%, is less viscous (by a factor of ~3 at low shear rate). As discussed in Section 4, reduction in coil volume, and hence in viscosity, is an expected consequence of conversion of 4-linked residues from $^4C_1$ to $^1C_4$ (Fig. 1), and has been demonstrated previously by calculation and experiment (Morris et al., 1978).

In contrast to the shear-thinning behaviour observed for 1.0 wt.% lambda carrageenan in water, the solution of 2.0 wt.% theta carrageenan in water is essentially Newtonian, showing no significant change in viscosity with increasing shear rate, which is again consistent with lower coil volume and hence less interpenetration (entanglement) of adjacent chains. The solution in 0.1 M

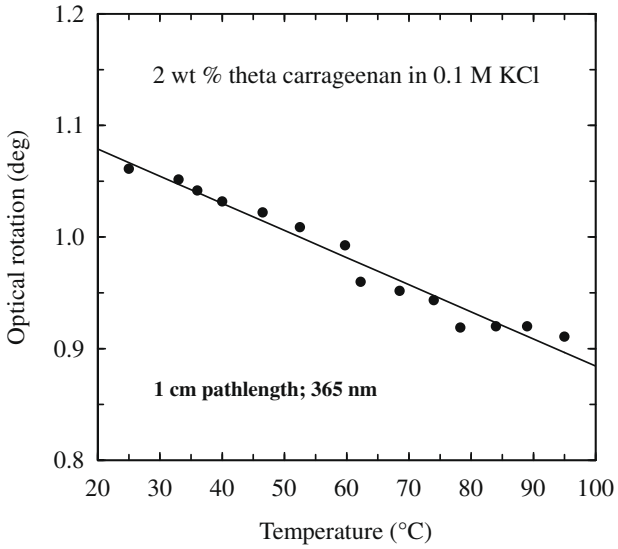

Fig. 3. Values of optical rotation (pathlength 1 cm; wavelength 365 nm) recorded on cooling for 2.0 wt.% theta carrageenan in 0.1 M KCl.

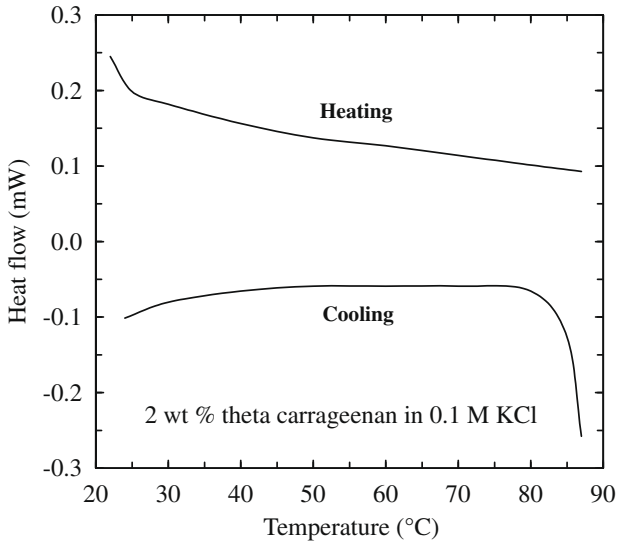

Fig. 4. DSC heating and cooling scans (0.3 °C/min) for 2.0 wt.% theta carrageenan in 0.1 M KCl.

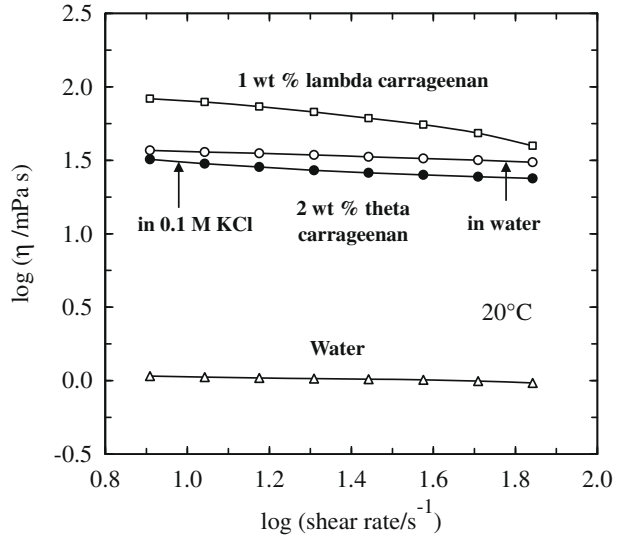

Fig. 5. Shear-rate dependence of viscosity (20 °C) for water (△), 1.0 wt.% lambda carrageenan in water (□), and 2.0 wt.% theta carrageenan in water (○) and in 0.1 M KCl (●).

KCl is also Newtonian, but with slightly lower viscosity at all shear rates. The difference can be explained by reduction in coil volume in response to screening of intramolecular electrostatic repulsion between segments of the polyelectrolyte chains as ionic strength is increased.

The studies of theta carrageenan by the steady-shear (rotational) measurements of viscosity reported above indicate behaviour typical of solutions of disordered polyelectrolyte coils. This conclusion was explored further by rheological studies using low-amplitude oscillation. In all of these experiments, storage modulus (G') was too low to be measured, even on the highly-sensitive rheometer used, which in itself argues against intermolecular association to form a crosslinked (gel) network.

The first step was to define the linear viscoelastic regime, within which moduli remain independent of amplitude of oscillation (Ross-Murphy, 1984). Fig. 6 shows the variation of G'' with applied strain for 2.0 wt.% theta carrageenan in water (at 20 °C). The

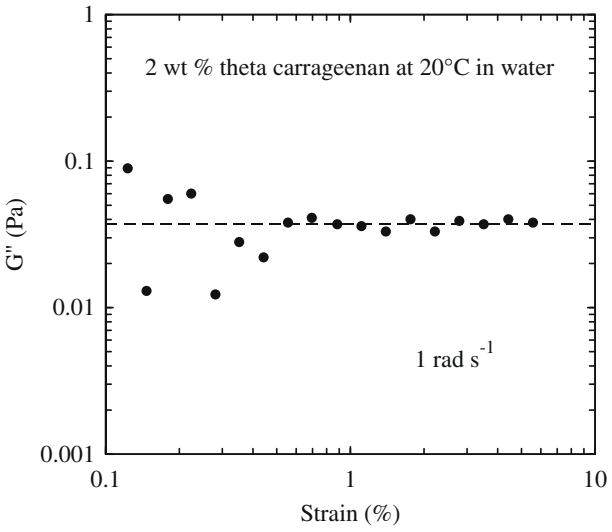

Fig. 6. Strain-dependence of G'' (1 rad s^{-1}) for 2.0 wt.% theta carrageenan in water at 20 °C.

modulus (at 1 rad s^{-1}) is essentially constant up to the highest strain applied (5.6%). A strain of 1% was used as a value well within the linear region.

Fig. 7 shows the variation of G'' (1 rad s^{-1}; 1% strain) for the same solution (2.0 wt.% theta carrageenan in water) on cooling from ~80 to ~10 °C. There is a small, steady increase in modulus with decreasing temperature, but no indication of the sharp increase that would be expected for intermolecular association by formation of double helices.

In the experiment leading to the results shown in Fig. 7, the solution was coated around its periphery with light (115 cS) silicone oil, to minimise evaporation at high temperature (which is standard procedure for heated samples). As shown in Fig. 8, the variation of log G'' with log ω recorded for the same sample at 20 °C was linear, with a slope of 1.0, which is typical (Ross-Murphy, 1984) of disordered coils at low frequencies where there is sufficient time for intermolecular entanglements to come apart within the period of oscillation. Another typical feature of rheology of disordered coils (Ross-Murphy, 1984), however, is that they obey the "Cox–Merz

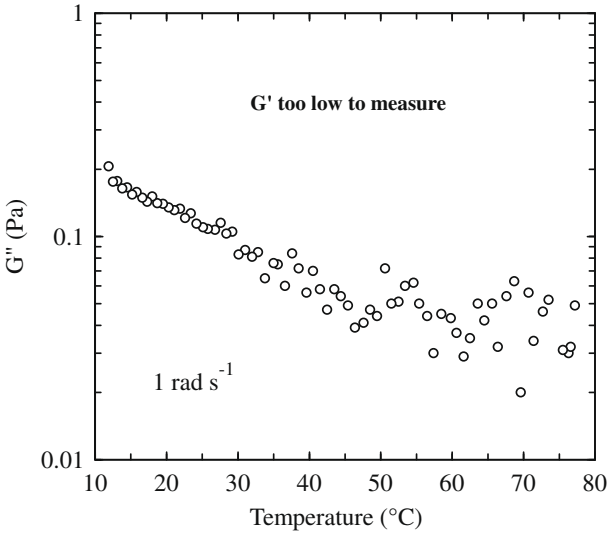

Fig. 7. Values of G'' (1 rad s^{-1}; 1% strain) recorded on cooling for 2.0 wt.% theta carrageenan in water.

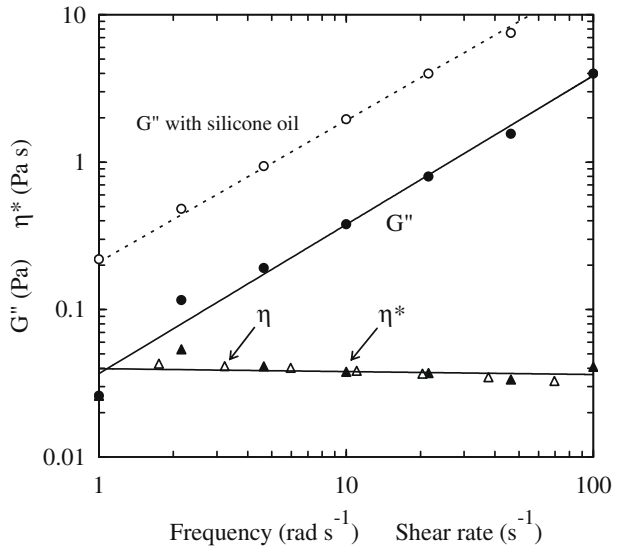

Fig. 8. Solution rheology of 2.0 wt.% theta carrageenan in water at 20 °C: frequency-dependence of G'' for samples with (○) and without (●) 115 cS silicone oil around their periphery; comparison of the frequency-dependence of η^* (▲) and shear-rate dependence of η (△) for samples without silicone oil.

rule" (Cox & Merz, 1958); i.e. their frequency-dependence of η^* and shear-rate dependence of η superimpose closely at equivalent values of frequency (ω/rad s^{-1}) and shear rate ($\dot{\gamma}/s^{-1}$). For the solution of 2.0 wt.% theta carrageenan (coated with silicone oil), however, η^* (at 20 °C) was substantially (~5 times) higher than the rotational viscosity (η) of the same sample (Fig. 5).

To explore this puzzling behaviour further, the oscillatory measurements (at 20 °C) were repeated, but without silicone oil. As shown in Fig. 8, the variation of log G'' with log ω was again linear with a slope of 1.0, but the absolute values were lower, and there was close Cox–Merz superposition of η and η^*. It is evident, therefore, that because of the very low modulus (G'') and dynamic viscosity (η^*) of theta carrageenan, even at the comparatively high concentration studied (2.0 wt.%), the contribution to overall rheology from low-viscosity oil around the periphery of the sample is appreciable. When this complicating factor is eliminated, however, the rheology of theta carrageenan is entirely typical of a solution of disordered coils.

4. Discussion and conclusions

The main conclusion from this investigation is that theta carrageenan, under conditions that would promote conformational ordering and gelation of iota and kappa, shows no evidence of forming double helices or network structure, but instead has the rheology typical of disordered polyelectrolyte coils. As discussed in Section 1, the absence of helix formation (and associated gelation) is an expected consequence of sulfation at O(2) of 3-linked residues.

The gelation of theta carrageenan reported by Thành et al. (2002) can probably be traced to the composition of the sample studied. In Table 2 of their paper, Thành et al. report a significant content of anhydrogalactose (AG) in the lambda carrageenan used for preparation of theta (1.0:0.14 Gal:AG), which, as shown in Fig. 1, suggests appreciable contamination with gelling carrageenan. Survival of this gelling material in the resulting theta carrageenan would offer a simple explanation of the gel structure reported in Table 4 of the paper by Thành et al. (2002) for 1.5 wt.% polymer at low temperature in 0.1 M KCl. The absence of any detectable contamination by gelling carrageenans in the theta carrageenan sample studied in the present work is demonstrated by the IR spectrum shown in Fig. 2b and discussed in Section 3.1.

The very low solution viscosity of theta carrageenan (Figs. 5 and 8) is consistent with general principles of polysaccharide chain geometry (Rees, 1973) established by computer modelling. The hydrodynamic volume of disordered polysaccharide coils increases as the stiffness of the polymer chains increases, with consequent increase in solution viscosity. Chain stiffness originates from restricted rotation of adjacent sugar residues around the glycosidic linkage. The two main factors (Rees, 1973) contributing to restriction of conformational freedom are (i) OH groups, or other bulky substituents, in equatorial positions on either side of the glycosidic bond, and (ii) axial linkages to the glycosidic oxygen, which bring the neighbouring residues into closer proximity to one another than equatorial attachment. Insertion of the anhydride bridge on alkali modification of lambda carrageenan suppresses both of these: the only equatorial groups in the resulting anhydrogalactose (Fig. 1b) are hydrogen atoms, and the diaxial linkage geometry of the 4-linked residues (Fig. 1a) is replaced by diequatorial (Fig. 1b), which explains why theta carrageenan gives less viscous solutions than its lambda carrageenan precursor. These qualitative considerations were quantified in the investigation by Morris et al. (1978), where increase in flexibility on alkali modification of lambda carrageenan was characterised by the ability of the polymer coils to contract in response to progressive screening of intramolecular electrostatic repulsion with increasing ionic strength, and found to correlate well with values of persistence length derived by conformational analysis.

Finally, from a practical standpoint, theta carrageenan obviously cannot be used as a gelling agent. However, because of its extremely low solution viscosity, it would also be of little use as a thickener, and is therefore unlikely to have any potential for commercial exploitation.

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