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CALORIMETRIC AND CHIROPTICAL EVIDENCE OF AGGREGATE-DRIVEN HELIX FORMATION IN CARRAGEENAN SYSTEMS

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ABSTRACT

Thermally induced, order–disorder transitions of iota- and kappa-carrageenan have been monitored by optical rotation and differential-scanning calorimetry in various ionic environments. Conformational ordering in kappa-carrageenan is observed only in the presence of cations that have been shown previously to promote helix–helix aggregation, and shows marked hysteresis between heating and cooling. Iota-carrageenan, by contrast, shows an order–disorder transition in the non-aggregating, tetramethylammonium salt form, at substantially lower temperature than for kappa-carrageenan, and without hysteresis. In the presence of potassium ions, which are known to promote aggregation, iota-carrageenan shows two distinct thermal-transitions, one without hysteresis at the same temperature as observed under non-aggregating conditions, and one with significant hysteresis close to the temperature of the kappa-carrageenan transition. We interpret these transitions as helix-to-coil and aggregated helix-to-coil, respectively. This interpretation is supported by measurements of the enthalpy changes of the transitions; ΔH values show a systematic increase with increasing aggregation and hysteresis. We conclude that the double helix of iota-carrageenan can exist as a stable entity in isolation, but may be further stabilised by aggregation, whereas the kappa-carrageenan helix is stable only when aggregated.

INTRODUCTION

The importance of ordered molecular structures of specific, fixed geometry in determining the physical and biological properties of proteins and polynucleotides is well-established. Over the last decade, it has been demonstrated that carbohydrate polymers show similar conformational behaviour, and the biological and technological utility of many polysaccharide systems has now been interpreted in terms of ordered, tertiary molecular structures[1–4]. The most extensively investigated of these is the carrageenan double-helix[5].

Carrageenans occur as the major polysaccharide component of several genera of Rhodophyceae (marine red-algae). The primary structure of the helix-forming members of this family is based[6,7] on an alternating repeating-sequence of 1,3-linked β-D-galactose 4-sulphate residues and 1,4-linked 3,6-anhydro-α-D-galactose residues, with various degrees of 2-sulphation of the anhydro ring (see Fig. 1). Iota-carrageenan approximates to a fully 2-sulphated structure, whereas 2-sulphation is essentially absent in kappa-carrageenan. X-Ray fibre-diffraction studies, with linked-atom refinement against measured diffraction intensities[8], show clear evidence of double-helix geometry for iota-carrageenan in the solid state. Diffraction photographs for kappa-carrageenan, although of poorer quality, showed striking similarities to those obtained for iota-carrageenan[9], and an analogous double-helix structure seems probable, although alternative models have yet to be rigorously excluded. For both polymers, conformational order persists under conditions of extensive hydration in solutions and gels, and thermally induced, order–disorder transitions have been characterised by a variety of physical techniques[10–14].

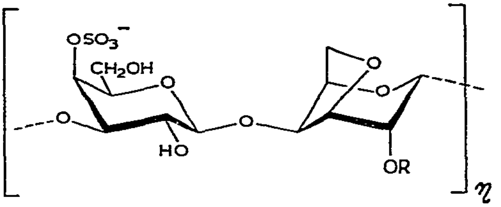


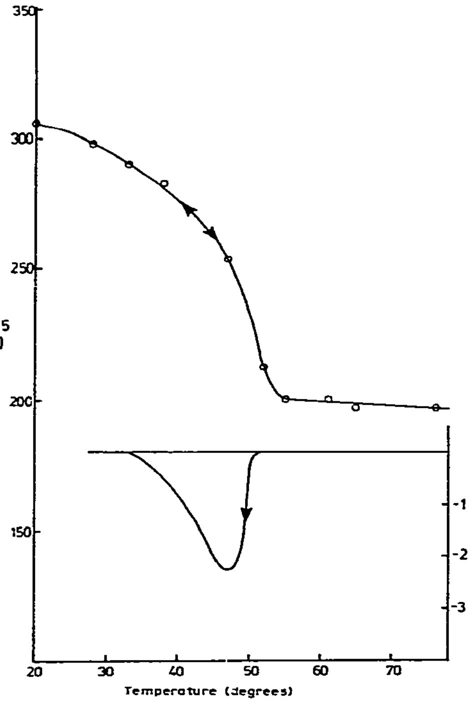
Fig. 1. Idealised disaccharide repeating-structure for carrageenan; iota, R = SO_3^-; kappa, R = H.

Conformational ordering may be accompanied by a sol→gel transition, but this is not invariably the case, and a detailed understanding of the molecular mechanism of gelation has only recently been developed[5]. For both iota- and kappa-carrageenan, optimum gelation with monovalent cations, as judged by the mechanical properties of the gels, is achieved with potassium or rubidium ions. Substantially weaker gels are formed in the presence of caesium or ammonium as sole counter-ion, and the bulky tetramethylammonium ion completely abolishes gel formation[5]. In the potassium salt form, the magnitude of the change in optical rotation on helix formation is closely similar for both iota- and kappa-carrageenan. Iota-carrageenan shows a comparable degree of conformational ordering in the presence of other monovalent cations, in particular sodium and tetramethylammonium. For kappa-carrageenan, however, helix formation is totally abolished on conversion into the tetramethylammonium salt form, while in the presence of sodium as sole counter-ion, limited helix-formation is observed only under forcing conditions of very low temperature and high ionic strength. Light-scattering measurements on structurally regular, short-chain segments of iota-carrageenan, which are capable of double-helix formation without gelation[10], show that cations which promote gelation of the intact polymer also promote helix aggregation in solution[5].

On the basis of this evidence, we have proposed a two-stage "domain" model of carrageenan gelation[5]. In terms of this model, interchain association through double helices is limited to the formation of small, soluble clusters ("domains") of about ten chains. Development of a continuous, three-dimensional network involves further association of these domains by cation-mediated, helix–helix aggregation, with specific incorporation of cations of appropriate geometry within the ordered, tertiary structure of the junction zone. We now present independent evidence for a two-stage gelation mechanism, from differential-scanning calorimetry (d.s.c.) and measurements of optical rotation. Under appropriate conditions of primary structure of carrageenan and cationic environment, the formation and melting of isolated and aggregated carrageenan-helices were found to be well-resolved thermal events.

EXPERIMENTAL

Materials. — Kappa-carrageenan (Rex 5401) was a commercial sample from Marine Colloids Inc. Structural regularity was improved by alkaline borohydride treatment to convert[15] anomalous galactose 6-sulphate residues into 3,6-anhydro-D-



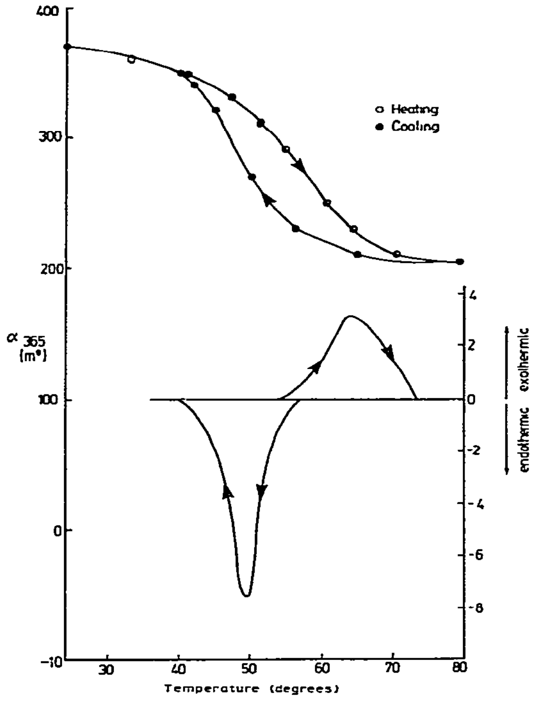


Fig. 2. Order–disorder behaviour of segmented iota-carrageenan (2.16% w/v) in the presence of tetramethylammonium (0.15M) as sole counter-ion, as monitored by optical rotation (upper curve; 365 nm, 1-cm pathlength) and d.s.c. (lower curve; arbitrary units).

Fig. 3. Order–disorder behaviour of kappa-carrageenan (1.84% w/v) in the presence of potassium (0.10M) as sole counter-ion, as monitored by optical rotation (upper curves; 365 nm, 1-mm pathlength) and d.s.c. (lower curves; arbitrary units).

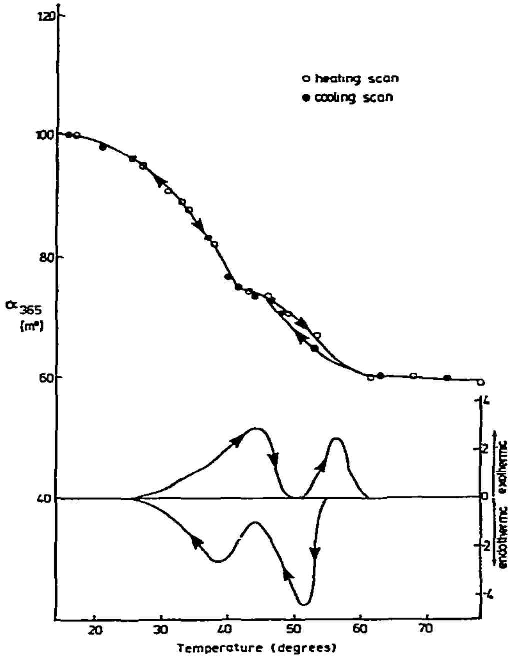
galactose residues. Iota-carrageenan (X52, Pierrefitte-Auby) was segmented by Smith degradation[16], and treated with alkaline borohydride as above. The samples were then extensively dialysed against deionised water, and specific salt forms were obtained by ion exchange on Amberlite IR-120 resin, followed by freeze-drying. Absolute concentrations were determined by elemental analysis of the freeze-dried material (Butterworth Microanalytical Consultancy Ltd.).

Methods. — Optical rotation was measured at 365 nm on a Perkin–Elmer 241 polarimeter, using 1-cm or 1-mm cells. Temperature was controlled by a Haake thermocirculator, and solutions were held at each temperature until a stable, equilibrium reading was attained. D.s.c. was performed with a Perkin–Elmer DSC-2 calorimeter. Most results were recorded at a scan rate of 10 $K.min^{-1}$, but were occasionally verified by measurements at 5 $K.min^{-1}$ or 20 $K.min^{-1}$. Heating and cooling curves for both optical rotation and d.s.c. were repeated several times for each of three separate samples. The measurements were very reproducible, and typical data points are shown at intervals along the best-fit curves in Figs. 2 and 3.

RESULTS AND DISCUSSION

Fig. 2 shows the temperature course of the order–disorder transition, as monitored by both optical rotation and d.s.c., for segmented iota-carrageenan in the presence of tetramethylammonium as sole counter-ion. Estimates of the transition midpoint from d.s.c. cooling-scans and from optical rotation agree within experimental error. D.s.c. heating-curves mirror the cooling scan, but with a 5-K shift to lower temperature. Kinetic studies[14] show that the transition is rapid compared with d.s.c. scanning-rates, and we therefore attribute the shift to thermal lag in the instrument and limitations of heat-transfer within the sample. Essentially identical results were obtained for the sodium salt form, which have previously been shown to be consistent with a simple two-state, all-or-none, order–disorder transition mechanism[13].

By contrast, kappa-carrageenan, in the presence of tetramethylammonium ions alone, shows no evidence of conformational ordering at any accessible temperature[5]. However, Fig. 3 shows the temperature profiles of optical rotation and d.s.c. for kappa-carrageenan in the gelling, potassium salt form. Both indices of conformational change display marked hysteresis in their temperature course. The d.s.c. curves are no longer mirror images after translation along the temperature axis, and the separation of their maxima is 14 K, 9 K greater than the thermal lag of the instrument. Similarly, there is an 8-K difference between the centres of the optical-rotation heating and cooling curves. In discussion of the sol→gel transition of agarose[17], we have argued that such hysteresis is indicative of aggregation, stabilising the helical structure to temperatures above those at which it would be stable in isolation. Comparison of the light-scattering behaviour of kappa-carrageenan segments in the random coil, sodium salt form and in the ordered potassium form provides direct evidence of extensive aggregation, since the observed increase in the



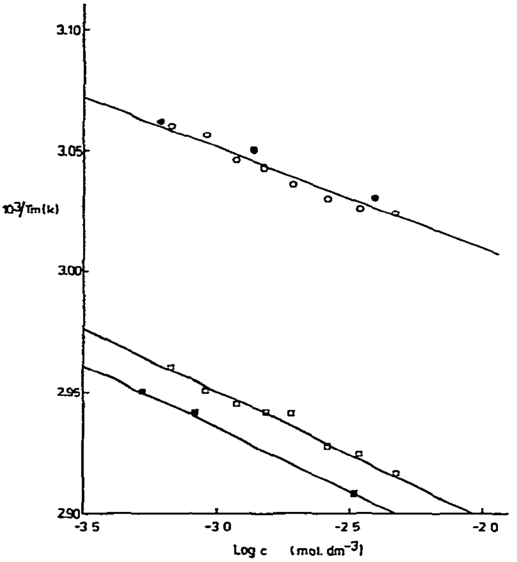


Fig. 4. Order–disorder behaviour of segmented iota-carrageenan (4.78% w/v) in the potassium salt form, as monitored by optical rotation (upper curves; 365 nm, 1-mm pathlength) and d.s.c. (lower curves; arbitrary units).

Fig. 5. Variation in order–disorder transition midpoint with carrageenan concentration (c), for kappa-carrageenan in the presence of 0.1M K^+ (■), segmented iota-carrageenan in the presence of 0.1M Me_4N^+ (●), and for the high-temperature (□) and low-temperature (○) transitions of segmented iota-carrageenan in the presence of 0.1M K^+.

molecular weight is several orders of magnitude greater than would be expected on the basis of a simple coil to double-helix mechanism[18].

Analogous, though far less-pronounced, aggregation effects are evident in the light-scattering behaviour of iota-carrageenan segments in the presence of potassium ions[5]. As shown in Fig. 4, this is dramatically reflected in the temperature course of the order–disorder transition. Both d.s.c. and optical rotation show clear evidence of two distinct molecular processes, separated in their transition midpoints by ~15 K. The event at higher temperature shows a degree of hysteresis which, although considerably less pronounced than for kappa-carrageenan, is well beyond experimental error. The temperature course of optical rotation for the lower-temperature process shows no hysteresis. The 5-K displacement in the midpoints of each transition between heating and cooling scans in d.s.c. is attributed to thermal lag, as discussed earlier.

In Fig. 5, the transition midpoints of these two processes at constant ionic strength (0.1 mol.kg^{-1}) and at various concentrations of carrageenan are compared with the corresponding temperatures for the single transitions shown by iota-carra-

TABLE I

ENTHALPY CHANGES FOR CARRAGEENAN ORDER–DISORDER TRANSITIONS

Carrageenan system	ΔH *(kJ.mol^{-1})*
Me_4N^+ Iota (0.1M Me_4NCl)	4.6 ±0.2
K^+ Iota (0.1M KCl)	5.0 ±0.3
K^+ Iota (0.25M KCl)	6.0 ±0.3
K^+ Kappa (1.84%; 0.1M KCl)	9.4 ±0.5
K^+ Kappa (4.04%; 0.1M KCl)	12.6 ±0.5

geenan in the non-aggregating, tetramethylammonium salt form, and kappa-carrageenan in the extensively aggregating, potassium salt form. Over the range of concentrations studied, the transition midpoint of the lower-temperature process is, within experimental error, identical to that of the simple, coil→helix transition in tetramethylammonium iota-carrageenan, while the temperature of the higher transition is within 2 K of that observed for potassium kappa-carrageenan. It appears, therefore, that the event at lower temperature is a simple coil→helix transition, as previously characterised, while the second transition is associated with the formation of larger molecular assemblies.

A comparison of enthalpy changes for the transitions, measured from the d.s.c. curves, provides further support for this interpretation. As shown in Table I, the enthalpy change for the coil→helix transition of tetramethylammonium iota-carrageenan is 4.6 kJ.mol^{-1}, irrespective of ionic strength or polysaccharide concentration. For potassium kappa-carrageenan, values 2–3 times higher than this are obtained, increasing with increasing concentration of polymer and potassium ions, both of which would be expected, by simple mass-action considerations, to promote aggregation. For potassium iota-carrageenan, intermediate transition-enthalpy values are obtained, which increase with increasing concentration of potassium, consistent with limited, cation-induced aggregation.

We conclude from these results that order–disorder transitions in carrageenan can occur by two different mechanisms, isolated helix→coil, and aggregated helix→coil. The latter process occurs at substantially higher temperatures than the former, has a considerably greater transition enthalpy, and shows pronounced thermal hysteresis. Our present results show that iota-carrageenan can react by either mechanism, depending on conditions of polymer concentration and ionic environment, whereas kappa-carrageenan appears to adopt the ordered helical conformation only under aggregating conditions. We therefore conclude that while the double helix of iota-carrageenan can exist as a stable entity in isolation, the kappa helix requires further stabilisation by aggregation, and can form only when the coil→helix transition is aggregation-driven. The close, quantitative similarities (Fig. 5) between the order–disorder behaviour of kappa-carrageenan and that of iota-carrageenan under aggregating conditions lends further support to previous suggestions[9,19] of analogous double-helix geometry in the ordered state.

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