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Preliminary communication

Evidence for a salt-promoted "freeze-out" of linkage conformations in carrageenans as a pre-requisite for gel-formation

OLAV SMIDSRØD, INGER-LILL ANDRESEN, HANS GRASDALEN, BJØRN LARSEN, and TERENCE PAINTER

Institute of Marine Biochemistry, N-7034 Trondheim-NTH (Norway)

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When the intrinsic viscosity, $[\eta]$, of an anionic polysaccharide is plotted against the reciprocal of the square root of the ionic strength of the solution, $I^{-0.5}$, straight-line plots of positive slope are usually obtained[1]. The slopes reflect the ease with which the chains coil up as their effective charge density decreases, and have been used to compare the stiffness of the chains in different anionic polysaccharides[1].

When this method was applied to a carrageenan isolated from *Eucheuma spinosum* ("iota carrageenan")[2], the unusual result shown in Fig. 1 was obtained. The stiffness appeared to increase with increasing ionic strength, and extrapolation of the linear parts of the curve to infinite ionic strength ($I^{-0.5} = 0$) indicated that the intrinsic viscosity of the effectively uncharged molecule was ~10 times greater than would be expected if the chains had contracted in the normal way.

To ensure that the effect was not due to the formation of insoluble particles of

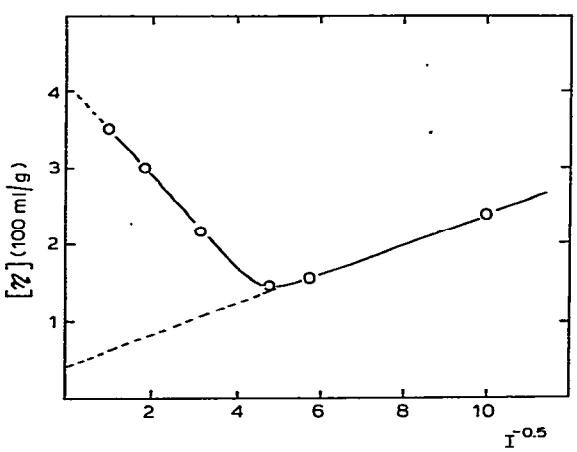

Fig. 1. Intrinsic viscosities, $[\eta]$, at 20° for iota-carrageenan, determined in a Ubbelohde No. I viscometer at different concentrations of lithium iodide. The iota-carrageenan was prepared by precipitation from an aqueous extract from *Eucheuma spinosum* with M KCl; ~80% of the material was precipitated at this ionic strength, the rest being soluble at this and all higher ionic strengths.

gel (microgels), the solutions were ultracentrifuged beforehand, and lithium iodide was used to vary the ionic strength, since aqueous solutions of this salt are good solvents for carrageenan gels. The change in stiffness must therefore have been due either to a conformational change in the isolated carrageenan molecules (*i.e.*, an intramolecular transition), or to a very limited, intermolecular association, such as a dimerisation.

Rees and his co-workers[3] have previously postulated the reversible formation of double helices by the potassium salt of a partially degraded sample of iota-carrageenan, under similar conditions. Since their arguments were based upon studies of the temperature-dependence of the specific rotation of the solutions, we have determined the temperature-dependence of the optical rotation at a series of different concentrations of lithium iodide, with the results shown in Fig. 2. It is seen that, at 20°, the optical rotation increases markedly with ionic strength, leaving little doubt that the increases in intrinsic viscosity and optical rotation with ionic strength have a common origin. Closely similar results were obtained for solutions in sodium or potassium chloride, and, hence, the phenomenon is not markedly ion-specific. In agreement with Rees *et al.*[3], we found no hysteresis in the temperature effect.

We have been unable to confirm that the observed phenomena were due to the formation of double helices, or to intermolecular association of any kind. Thus, the changes in optical rotation were essentially unchanged by degradation of the samples to a $\overline{d.p.}_n$ of 50, which would not be expected for a co-operative winding-up of the chains into double helices[3]. Moreover, in the absence of hysteresis, such an association would imply that a

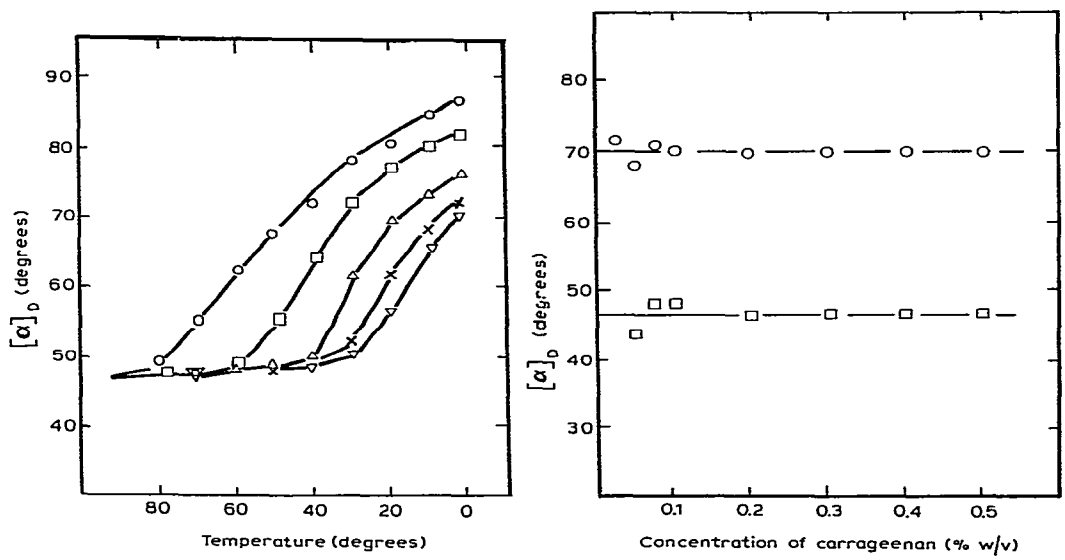

Fig. 2 (left). Specific optical rotation for a 3% (w/v) solution of iota-carrageenan at different temperatures and ionic strengths in lithium iodide: o, M; □, 0.3M; △, 0.1M; ×, 0.03M; ▽, 0.01M.

Fig. 3 (right). Specific rotation of iota-carrageenan at different concentrations of carrageenan: o, 30°; □, 60°. The range of concentrations shown is much lower than that in Fig. 2, but concentration-independence of $[\alpha]_D$ was observed upto 15% (w/v) carrageenan.

range of temperatures should easily be found in which there is a strong dependence of specific rotation upon the concentration of carrageenan. For dimerisation of short chains, the expected dependence would be approximately of the second order. However, no such dependence was found at any temperature or salt concentration, and Fig. 3 shows the results of experiments carried out at 30 and 60° (*cf.* Fig. 2) in 0.3M lithium iodide. Measurements of number-average degrees of polymerisation ($\bar{M}_n$) were also carried out at different temperatures, and Table I shows some results obtained with samples of iota-carrageenan that had been degraded, with mild acid, to different extents. The conformational transition must therefore be intramolecular.

TABLE I

NUMBER-AVERAGE MOLECULAR WEIGHTS[a], $\bar{M}_n$, FOR IOTA-CARRAGEENANS IN 0.3M LITHIUM IODIDE AT 30 AND 60°. THE THREE SAMPLES WERE PREPARED BY DEGRADATION, AT 80° AND pH 2, FOR DIFFERENT TIMES. THEIR SPECIFIC ROTATIONS WERE THE SAME, TO WITHIN ±2°, AS THOSE REPORTED IN FIG. 3 FOR UNDEGRADED IOTA-CARRAGEENAN AT THE CORRESPONDING TEMPERATURES

Time of degradation (min)	$\bar{M}_n$	
	30°	60°
10	63000 ±10000	63000 ±10000
18	41000 ±6000	52000 ±6000
60	16000 ±3000	15000 ±3000

[a] Determined in a Knauer membrane osmometer.

The precise nature of the transition is still unclear, but preliminary ^{13}C-n.m.r. data do not suggest any change in the ring conformations of either the β-D-galactopyranosyl or the 3,6-anhydro-α-D-galactopyranosyl residues. The spectra (Fig. 4) indicate that, upon cooling the solution from 90 to 25°, three broad signals originating from C-1 of the two monomeric units and from C-3 of the D-galactopyranosyl residues appear, in addition to the three narrower peaks situated at slightly higher fields and originating from the same three carbon atoms[4]. This finding is most simply explained as an ordered conformation, existing together with a random coil, at the lower temperature. It seems most likely that the ordered conformation is one in which the rotameric forms of the glycosidic linkages (*i.e.*, "linkage conformations") have become "frozen" in a particular arrangement. It may be supposed that, at low ionic strength, the mutual electrostatic repulsion between neighbouring $-OSO_3^-$ groups is sufficiently strong to over-ride steric preferences, which, however, again become dominant as the ionic strength increases. Apart from an apparent effect upon the proportion of aldehydo-form in reducing, terminal 3,6-anhydro-D-galactose residues, the ^{13}C-n.m.r. spectra were closely similar in the presence of lithium iodide, sodium chloride, and potassium chloride, thus confirming that the transition is not markedly ion-specific.

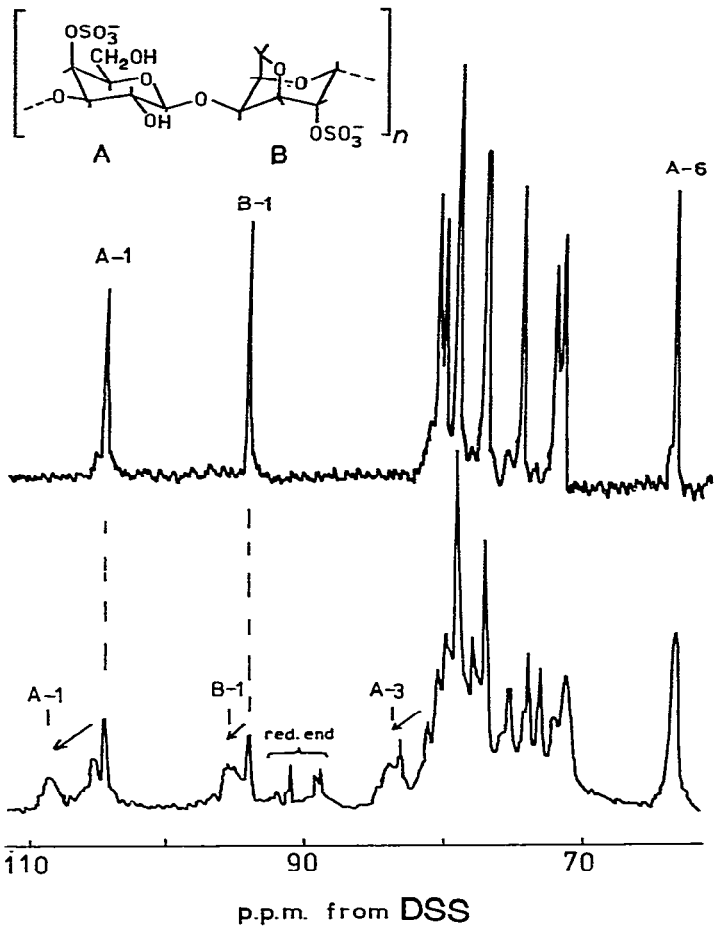

Fig. 4. 25-MHz proton-decoupled ^{13}C-n.m.r. spectra of iota-carrageenan (12%, w/v) in M LiI at different temperatures: top, 90°, $\overline{d.p.}_n$ 20–30; bottom, 25°, $\overline{d.p.}_n$ 6–10. Three downfield-shifted, broad ^{13}C-resonances in the lower spectrum are assigned to linked carbons as indicated.

In apparent contrast with these findings, the formation of gels by κ-carrageenan is highly ion-dependent, the most marked effect being that observed when the counterion is changed from sodium or lithium to potassium, rubidium, or caesium[5]. Nevertheless, we have found that, for solutions in aqueous lithium iodide, κ-carrageenan and its fragments show all the changes noted here for the iota-carrageenan. The two carrageenans differ mainly in sulphate content, and κ-carrageenan is inherently the less soluble in water, in the presence of any salt. Several workers[6–8] have pointed out that the solubility of any polyanion should be affected by the extent to which its counterion is hydrated, and that the hydration of alkali-metal ions diminishes sharply with increasing atomic number.

Gel formation may therefore be seen as a two-step process, the first involving an intramolecular, conformational change and the second involving a decrease in solubility that is ion-dependent.

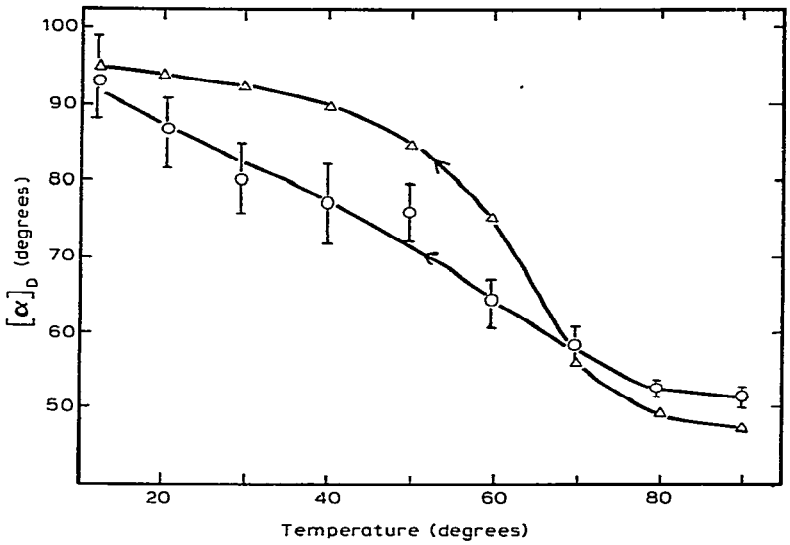

Fig. 5. Specific optical rotation of 3% (w/v) aqueous κ-carrageenan at different temperatures: △, 0.3M LiI; ○, 0.3M KCl. More than 90% of the extract from *Eucheuma cottonii* precipitated sharply between 0.03 and 0.06M KCl, and the extract was used without purification.

The voluminous nature of potassium carrageenate gels does not suggest any close association between chains, but rather a certain incompatibility that opposes close-packing into a fibre. It is evident that the ordered conformation that is formed in the first step does not readily close-pack, but it remains to be shown whether this conformation is retained in the gel state, or whether there is a second, major conformational change (such as double-helix formation) when the gel is formed. To test this idea, the temperature-dependence of the specific rotation of aqueous lithium and potassium κ-carrageenates prepared from *Eucheuma cottonii*[2] was examined (Fig. 5). The former was a true solution throughout, while the latter was a firm gel below 50°. The turbidity of the gel impaired accuracy considerably, but the results show little difference in optical rotation between the sol and gel states. It is therefore unlikely that double-helix formation is an important feature of the gelling mechanism.

The actual nature of the intermolecular contact zones in potassium carrageenate gels remains to be established, and they are possibly of more than one kind. However, we have observed that some sites in gels of κ-carrageenan show marked selectivity for binding of potassium ions. The selectivity coefficient[9], $K^{K^+}_{Na^+}$, at low potassium contents, was ~10 for κ-carrageenan, compared to ~2–3 for iota-carrageenan gels. This finding suggests the formation of ion-selective "salt bridges", as proposed by Bayley[10].

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