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ON THE SPECIFICITY OF THE BINDING OF CATIONS TO CARRAGEENANS: COUNTERION N.M.R. SPECTROSCOPY IN MIXED CARRAGEENAN SYSTEMS*

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ABSTRACT

Counterion n.m.r. relaxation rates and signal intensities from mixed ion forms (sodium and rubidium) and mixed types (kappa and iota) of gel-forming carrageenans are presented. Data from the gel-promoting rubidium ion and the inert sodium ion obtained in the same mixed kappa-carrageenan systems indicate that only the rubidium ion binds to kappa-carrageenan and only in the gel state. Under non-gelling conditions, the transverse and longitudinal relaxation rates of sodium and the longitudinal relaxation rate of rubidium are independent of the ionic composition of samples of kappa-carrageenan.

In systems containing mixed iota/kappa-carrageenan of the rubidium form, the large line-broadening effects observed for rubidium correlate with the kappa-carrageenan transition, which occurs in a temperature range well separated from that of the iota-carrageenan transition. The same conclusion holds for natural iota-carrageenan (from *Euchema spinosa*), where a small fraction of kappa-carrageenan is responsible for the large effects of ion binding observed in the rubidium relaxation.

INTRODUCTION

The carrageenans[1] are linear sulfated galactans, extracted from certain marine red algae, which are widely used and studied because of their ability to act as thickeners or form aqueous gels. Different types of carrageenans may be distinguished by their primary structure, and the gel-forming iota- and kappa-carrageenans are dominated by regular sequences[1,2] of the disaccharide repeating-units **1** and **2**, respectively. The thermoreversible gelation of iota- and kappa-carrageenan involves a coil-to-helix transition of these regular sequences, which may be accompanied by aggregation of the helical molecules to infinite network structures[2].

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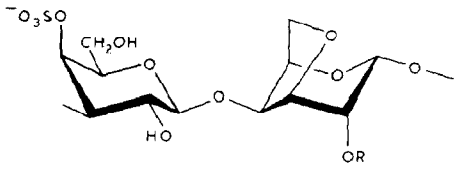

1 R = SO_3^- (iota)
2 R = H (kappa)

Both kappa- (**2**) and iota- (**1**) carrageenan are polyions and, consequently, salts affect their conformational transitions and gelation behavior[3–21]. However, particularly conspicuous in the case of carrageenans is a dramatic cation specificity[3–6,8,9] in their conformational transitions and association processes, which is also reflected in the rheological properties[8,12–19]. Thus, for the different ion forms of kappa-carrageenan[5], some "specific" monovalent cations (*i.e.*, rubidium, potassium, and cesium) induce the formation of helices at concentrations between one and two orders of magnitude lower than that of "inert" cations (sodium, tetramethylammonium, and lithium). The non-specific electrostatic effects on the coil–helix transitions of kappa- and iota-carrageenan in the presence of inert monovalent and divalent cations are well described by the Poisson–Boltzmann cell model[20,21], but the specific effects of certain cations are less well understood. Although the binding of specific cations has been demonstrated for kappa-carrageenan by studies[15,22–24] of n.m.r. chemical shifts, the importance of this binding for the formation of gels by carrageenans has been questioned[13,24,25]. Moreover, it is not clear to what extent the specific binding of ions is sensitive to the conformation of the carrageenan molecule. Cation shift effects, indicative of ion binding accompanied by partial dehydration, occur only in the presence of the helical conformation of kappa-carrageenan, while ultrasonic absorption[6] and optical rotation[26] effects have been taken to indicate cation-specific interactions also with the coil conformation (in one instance[26] also for iota-carrageenan). It has been proposed[6] that the intrinsic cation selectivity of kappa-carrageenan (regardless of its conformation) is solely responsible for the observed effects of specific cations in this system.

Specific effects of cations on rheological properties[8,12,14] and ion binding[14,26] of iota-carrageenan have been found, although they are less pronounced than for kappa-carrageenan. Thus, in early systematic studies of different ion forms of iota-carrageenan[8,9], a hysteresis in the thermally induced conformational transition of the potassium form was found, indicating the formation of aggregates. Under certain conditions, two discrete steps in the transition of the potassium form could be found[8], namely, a reversible transition that occurred at the same temperature as the transition of the tetramethylammonium form and an ion-specific transition that occurred, with significant thermal hysteresis, at higher temperatures. It was proposed that the aggregation of iota-carrageenan helices was a specific process that occurred only in the presence of certain cations[8]. However, these studies were

performed on samples containing large proportions of the kappa-structure[9]. In recent studies[27] of natural iota-carrageenan samples of greater structural purity, no hysteresis effects were found and the dependence of the transition temperature on the identity of the cation was quite weak, in contrast to kappa-carrageenan[5]. These findings pointed to the possibly important role of kappa-carrageenan impurities in the cation-specific properties of iota-carrageenan samples.

In order to clarify the molecular origin of cation specificity in carrageenans, the method of counterion spin relaxation has here been used. This method is a very sensitive indicator[14,22,24,25] of cation-specific effects in carrageenan systems, and correlations[14,24,25] between gel formation and the broadening and loss of signal intensity in the n.m.r. spectra of the gel-promoting cations have been demonstrated. However, even in the absence of binding, line-broadening of the n.m.r. signals of small molecules in polysaccharide gels may be expected[28]. The first objective of the present study is, therefore, to investigate to what extent large relaxation effects of gel-promoting ions in carrageenan gels are, in fact, due to ion binding. The second purpose is to test further the suggestion[27] that the cation-specific effects often found for iota-carrageenan have their origin in small amounts of kappa-carrageenan impurities, which are generally present in natural extracts of iota-carrageenan[29]. In order to obtain this information, mixed ion forms and mixed types of carrageenan have been studied, whereby possible interpretational ambiguities may be avoided.

EXPERIMENTAL

Materials. — Commercial samples of iota- (from *Euchema spinosa;* Lot No. 124F-0605) and kappa-carrageenan (from *Euchema cottonii;* Lot No. 124F-0604) were obtained from Sigma. The kappa-carrageenan was dissolved in water at elevated temperatures, 1 vol. of 0.3M KCl was added, and the mixture was cooled, with stirring, in an ice–water bath. The resulting gelatinous precipitate was collected by centrifugation at 10,000 r.p.m. for 30 min, and subjected again to the dissolution–precipitation–centrifugation procedure. The resulting gel was dissolved in water and precipitated by the addition of 2 vol. of ethanol, washed with aqueous 75% ethanol (3 times), aqueous 96% ethanol (3 times), and ether (3 times), and then dried overnight. A similar procedure, but with 3M KCl in the initial precipitation step, was used for iota-carrageenan. Pure ion forms of the carrageenans were obtained by ion exchange at elevated temperatures, followed by freeze-drying. Segments of enhanced structural regularity, prepared from the untreated commercial samples as described by Bryce *et al.*[30], were dialyzed against millipore-filtered water and freeze-dried.

Samples for n.m.r. measurements were made by adding the appropriate solvent (water or salt solution) to known amounts of freeze-dried carrageenan in a soda-glass tube (of a size which fitted snugly into standard 12-mm n.m.r. tubes), which was sealed and heated, with occasional shaking, in boiling water until a clear homogeneous solution was obtained. Ideal disaccharide molecular weights of the

freeze-dried pure ion forms were assumed in the calculation of the molar (disaccharide) concentrations of carrageenan in the samples.

Carrageenan segments[30] have been shown[27] (by proton n.m.r. spectroscopy) to contain structural impurities[29]. The iota-carrageenan used here contained ~10% of kappa-structure and *vice versa*. Values of the fractional content of kappa-carrageenan of the mixed samples are given in weight per cent of the dry material and refer to the amount of material originating from *E. cottonii* rather than to the actual content of kappa-structure, which is not known accurately.

N.m.r. spectroscopy. — A Nicolet NIC-360 spectrometer was used, operating at 118.38 and 95.70 MHz for the ^{87}Rb and ^{23}Na nuclei, respectively. Acquisition parameters were as follows:

Nucleus	*$\pi/2$ pulse length (μs)*	*Sweep width (Hz)*	*Points acquired*	*Dwell time (μs)*
^{23}Na	38–42	3000	1024	333
^{87}Rb	25–42	40,000	1024	25

Longitudinal relaxation rates (R_1) were obtained from inversion recovery experiments. Line-widths (at half amplitude), $\Delta\nu$, were obtained from lorentzian fits to the absorption signals. Both ^{87}Rb and ^{23}Na are spin 3/2 nuclei and give rise to bi-exponential relaxation (bi-lorentzian spectra) under non-extreme narrowing conditions[31] (*i.e.*, when $R_2 > R_1$). Therefore, the line-width data reported were not converted generally into transverse relaxation rates (using the relation $R_2 = \pi\Delta\nu$). In some experiments, the rapid (R_2^+) and slow (R_2^-) rates of the two-component transverse relaxation were obtained by bi-exponential fits (using the theoretical[31] 3:2 ratio of the amplitudes of the slow and the fast components) to the free induction decays obtained experimentally (extrapolated to full intensity at time zero, where the dead time of the spectrometer was taken into consideration). This fitting procedure gives more weight to the determination of the slow-relaxing component and the effects of second-order dynamic frequency shifts[32] are neglected. The R_2^+ values reported should therefore not be regarded as quantitative. For ^{23}Na, contributions from magnetic field inhomogeneity to the line-widths/transverse relaxation rates were significant. Such contributions (~3 Hz) were evaluated separately in each experiment (from comparisons of line-width and R_1 measurements on reference salt solutions) and are subtracted from the sodium results. In order to remove the trivial dependence of the measured relaxation data on temperature and to facilitate comparison between data from the two investigated ions, only normalized (with respect to the reference solutions) relaxation data are reported. Reported signal intensities were obtained by comparing areas of the absorption signals of the samples with those of reference salt solutions recorded under identical conditions. The peak areas were obtained either by integration (Fig. 1) or by excision and weighing (Figs. 5 and 6).

Optical rotation data. — These were obtained for a 5-cm pathlength with a Jasco DIP-360 polarimeter. Values of the specific optical rotation, $[\alpha]$, are given in degrees.cm^{-1}.M(disaccharide)$^{-1}$.

RESULTS AND DISCUSSION

General considerations. — The n.m.r. relaxation behavior and, consequently, the line-shapes of quadrupolar ions in gel-forming polymer systems may, in general, be quite complex where the effects of diffusion in ordered systems[33,34] and, possibly, (slowly exchanging) site-bound ions are involved. However, a common feature of ion n.m.r. in these types of systems[35,36] is that the nuclei experience different environments on the time scale of n.m.r. relaxation and, consequently, the observed n.m.r. relaxation rate will be an average over these environments, or states. For a site-binding macromolecular system, one may, in the simplest case, write the observed relaxation rate as a population-weighted average[37] so that, when only two states have to be considered, equation *1* obtains:

$$R_{i,obs} = p_B R_{i,B} + p_F R_{i,F}, \qquad 1$$

where $R_{i,obs}$ is the observed relaxation rate and p_B and p_F are the fractions of ions residing in the bound and free states, respectively, the two states being characterized by the intrinsic relaxation rates $R_{i,B}$ and $R_{i,F}$. Relation *1* is valid only when the exchange between the two states is rapid compared to the intrinsic relaxation rates. However, this restriction is essentially troublesome only in the interpretation of a relaxation enhancement due to ion binding in terms of p_B and $R_{i,B}$, which will not be attempted here. A more serious problem is that, in aggregating macromolecular systems, the relaxation rate $R_{i,F}$ is not, in general, equal to the relaxation rate in a simple salt solution, since non-specific electrostatic interactions[33–36,38] and the size, shape, order, and rigidity of macromolecules or macromolecular aggregates[33,34,38] may enhance the relaxation also for the free ions. Thus, enhancement of the relaxation of an ion in a macromolecular system does not necessarily indicate ion binding.

The general features of counterion n.m.r. relaxation in solutions of non-aggregated polyelectrolytes are well known[35,36] and well understood[38]. For systems that are sufficiently concentrated, the relaxation of the free ions is unaffected by slow diffusion processes[38] and the extreme narrowing condition obtains. The enhancement of the counterion relaxation in such systems is dominated by the perturbation of the environment of ions residing within a distance of the order of the diameter of a hydrated ion from the polyion. In the absence of site-binding of ions,

$$R_{i,obs} = p_\delta R_{i,\delta} + (1 - p_\delta) R_{i,ref} \qquad 2$$

equation *2* may be used for the observed relaxation rate, where p_δ is the fraction of

ions perturbed by the polyelectrolyte and the local relaxation rate, $R_{i,\delta}$, differs from the bulk value, $R_{i,ref}$. With p_δ estimated from the Poisson–Boltzmann cell model and with $R_{i,\delta}$ used as a (constant) fitting parameter, equation *2* has been employed to rationalize the variation in counterion relaxation in polyelectrolyte titration[39] and ion competition[39,40] experiments. Thus, in a non-aggregating polyelectrolyte solution and in the absence of site-binding, the excess relaxation rate of the counterions, defined in equation *3*, is a measure of the fraction of counterions residing in the immediate vicinity of the polyelectrolyte.

$$R_{i,ex} \equiv R_{i,obs} - R_{i,ref} \qquad 3$$

Systems with mixed counterions. — An advantage of the n.m.r. method is that it allows the behavior of different molecular species in a mixed system to be monitored individually. In the present carrageenan systems, the gel-promoting rubidium ion and the inert sodium ion have been compared. Fig. 1 compares the intensities of the signals of ^{87}Rb and ^{23}Na as functions of temperature, in heating experiments, in salt-free systems of 64mM kappa-carrageenan with mixed counterions. The molar fraction of rubidium, x_{Rb}, was varied between 0 and 1 in increments of 0.2. There are large differences in the results from sodium and rubidium, with dramatic line-broadening effects[22,24,25] occurring only in the rubidium signals. The

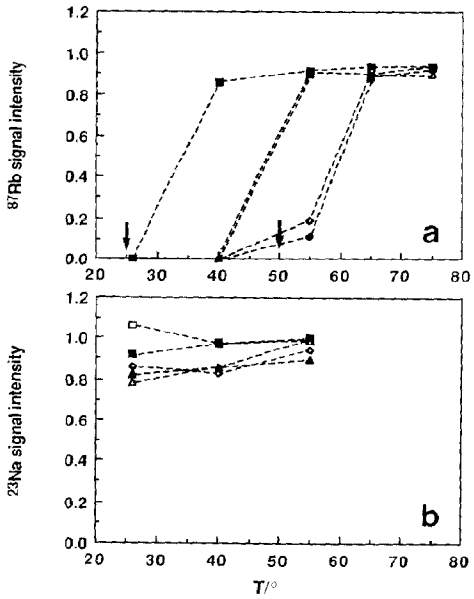

Fig. 1. Relative intensities of the n.m.r. signals of (a) $^{87}Rb^+$ and (b) $^{23}Na^+$ as functions of temperature in salt-free 64mM mixtures of Na and Rb carrageenan. Molar fractions of the rubidium form: 0 (□), 0.2 (■), 0.4 (△), 0.6 (▲), 0.8 (◇), and 1 (◆). Arrows indicate the temperatures of onset of helix formation, on cooling, in the samples with x_{Rb} 0.2 (left) and 1 (right).

intensity of the n.m.r. signal of the inert sodium ion was not significantly reduced in kappa-carrageenan even under gelling conditions, where the high resolution signal of rubidium was totally lost. These results indicate qualitatively different interactions of the sodium and the rubidium ions with kappa-carrageenan in the ordered (aggregated) state.

The intensities of the rubidium signal correlated with the macroscopic state of the system. The samples that showed full intensity were fluid, whereas the gel-like samples gave very broad ^{87}Rb signals. For example, the line-width for the pure rubidium form of kappa-carrageenan at 40° was 2–3 kHz (*cf.* 110 Hz for the reference solution of RbCl). The correlation[22,24,25] between gel formation in kappa-carrageenan and a dramatic broadening of the n.m.r. signal of the gel-promoting cations thus holds also in systems containing mixed cations. The differences in melting temperatures of the gel for the various cation forms correlated also with the counterion dependence[6] of the kappa-carrageenan order–disorder transition. Thus, the arrows in Fig. 1a indicate the temperatures of the onset of helix formation, as determined by optical rotation, in the samples with x_{Rb} 0.2 and 1, respectively. No conformational transition occurred in the sample with x_{Rb} 0 in the temperature interval 0–80°. The fact that the loss in intensity of the rubidium signal persisted at temperatures that are higher than that of onset of the formation of helices is a reflection of the thermal hysteresis of the helix–coil transition of kappa-carrageenan under aggregating conditions.

In the cases where full intensities of the n.m.r. signals were obtained, the relaxation rates of the ions in the mixed ion forms of kappa-carrageenan were also measured. Fig. 2 shows normalized line-widths and longitudinal relaxation rates for the sodium ion, at different temperatures, *versus* the fractional content of rubidium. It is clear that the data for the gels are distinctly different from those for fluid samples. In the fluid samples, the normalized relaxation rates of sodium were constant but slightly larger than those for the reference salt solution, regardless of temperature or ionic composition. Furthermore, under non-gelling conditions, the normalized longitudinal relaxation rates and the normalized line-widths of the n.m.r. signals are equal, indicating that the extreme narrowing condition obtained and that the line-width is a measure of the single transverse relaxation rate R_2, according to the relation $\Delta\nu = R_2/\pi$. The observation that the temperature dependence of the counterion relaxation in solutions of kappa-carrageenan scales with that of a simple salt solution is characteristic of purely electrostatic ion–polyion interactions, as the concentration profiles of ions in aqueous polyelectrolyte systems are insensitive to temperature[41]. The observation that the relaxation of sodium ions in *non-gelling* samples was independent of the rubidium content indicates that rubidium ions are not enriched preferentially in the vicinity of the kappa-carrageenan coil.

In contrast to the fluid samples, the kappa-carrageenan gels showed a dependence of the normalized relaxation data for the sodium ions on both the temperature and the rubidium content (Fig. 2). Furthermore, the relaxation rates were

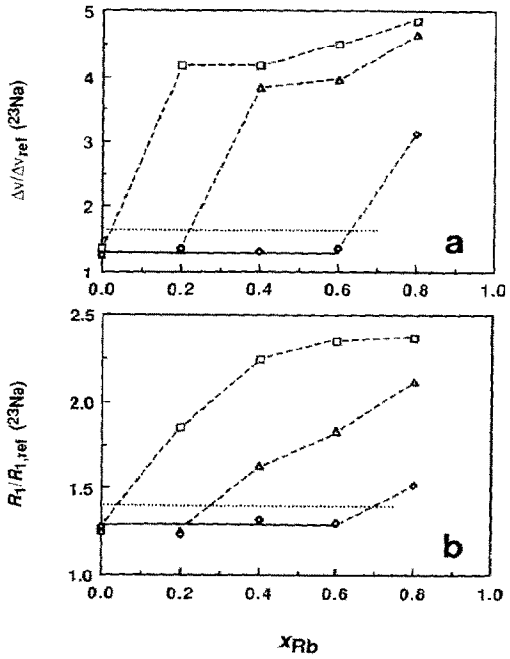

Fig. 2. Normalized (a) line-widths and (b) longitudinal relaxation rates of $^{23}Na^+$ in salt-free 64mM mixtures of Na and Rb carrageenan as functions of the molar fraction of the rubidium form obtained at 26° (□), 40° (△), and 55° (◇). Data points above the horizontal dotted lines refer to gel samples. Solid lines indicate the average value of all normalized relaxation rates (line-widths and R_1 data) obtained for fluid samples.

enhanced and the extreme narrowing condition was no longer satisfied. With the exception of the sample with x_{Rb} 0.8 at 55°, the n.m.r. signals were also markedly bi-lorentzian. Since rubidium binds specifically to carrageenan gels, the *increase* in the relaxation rates of sodium with increasing content of rubidium and decrease in temperature must reflect structural changes in the kappa-carrageenan gel. An enhancement of the relaxation rates of inert ions like sodium has been observed also in agarose gels[28,42], and the results in Fig. 2 are not evidence of any specific binding of sodium ions but a structure effect which seems to be general for all aggregated gel systems. The relative effect was larger in kappa-carrageenan than in agarose, but this may be due to the increased concentration of sodium ions close to the charged kappa-carrageenan molecules.

Data for the relaxation of rubidium in mixed ion forms of kappa-carrageenan in the fluid state are shown in Fig. 3, as normalized line-widths (only the spectrum of the sample with x_{Rb} 0.6 at 55° was visibly bi-exponential) and longitudinal relaxation rates. The longitudinal relaxation rates (Fig. 3b) of rubidium in the fluid samples conform to the picture seen with sodium, although the normalized rates were slightly larger for rubidium, *i.e.*, they were independent of temperature or

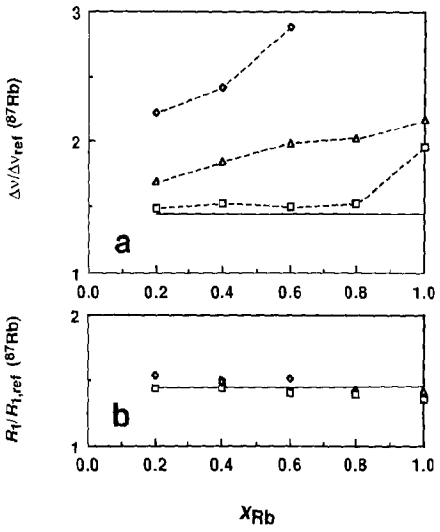

Fig. 3. Normalized (a) line-widths and (b) longitudinal relaxation rates of $^{87}Rb^+$ in salt-free 64mM mixtures of Na and Rb carrageenan as functions of the molar fraction of the rubidium form obtained at 55° (◇), 65° (△), and 75° (□). All data refer to fluid samples. Solid lines indicate the average value of the normalized longitudinal relaxation rates.

ionic content of the system, indicating that the interaction of rubidium ions with the kappa-carrageenan coil is of a non-specific electrostatic nature.

In contrast, the results in Fig. 3a show substantial, counterion-dependent line-broadening effects for the rubidium ion except for samples in the range $0.2 \leqslant x_{Rb} \leqslant 0.8$ at 75° where the extreme narrowing condition obtained. The significant differences between the rubidium R_1 and line-width data in most of the fluid samples may be due to a small fraction of aggregated kappa-carrageenan which persisted on heating, as kappa-carrageenan solutions must be heated to quite high temperatures[43] in order to obtain molecularly disperse systems. Owing to the extremely large rubidium line-broadening effects caused by ions bound to aggregates of kappa-carrageenan, very low concentrations of such aggregates will cause effects of the magnitude observed in Fig. 3a. These line-width results thus indicate that the samples had to be heated to ~75° before all rubidium binding structures had disappeared. The existence of few, specific binding sites is the most plausible explanation of these results, since any ion-specific interaction involving a significant proportion of the rubidium ions would have been evident also in the longitudinal relaxation rates. Furthermore, the *increase* in the line-width with increasing rubidium content shows that the line-broadening cannot be due to site-binding of rubidium ions to the kappa-carrageenan *coil*.

The results (data not shown) obtained for salt-free samples of iota-carrageenan with mixed cations were similar to those obtained by Belton *et al.*[14,25] for the pure ion forms. Thus, there were much larger relaxation effects for the rubidium ion than for the sodium ion in the gel state. The relaxation of rubidium was bi-exponen-

tial at lower temperatures, resulting in a maximum loss of signal intensity of ~40%, whereas there was no loss of intensity in the sodium signal although, as for kappa-carrageenan, a significant line-broadening in gel samples was observed. The samples with mixed cations behaved much like the corresponding single cation samples, except that the melting temperature of the gel, and the temperature at which substantial effects on the line shape of the rubidium signal were evident, varied with the fractional content of rubidium.

As for kappa-carrageenan, in the samples of iota-carrageenan with the highest content of rubidium, effects on the line shape of the rubidium signal were observed at temperatures well exceeding the gel melting temperature. These effects persisted at temperatures exceeding by >10° that at which complete conversion of iota-carrageenan into the coil conformation occurred. However, it was considered unlikely that the enhanced relaxation of rubidium in iota-carrageenan solutions was due to aggregates of *iota*-carrageenan persisting at high temperatures since no hysteresis of the transition, indicating aggregation, had been found for pure iota-carrageenan of any (monovalent) cation form[27]. Rather, the cation-specific effects found for the iota-carrageenan sample might derive from *kappa*-carrageenan impurities. The relaxation of rubidium in mixtures of iota- and kappa-carrageenan was therefore studied.

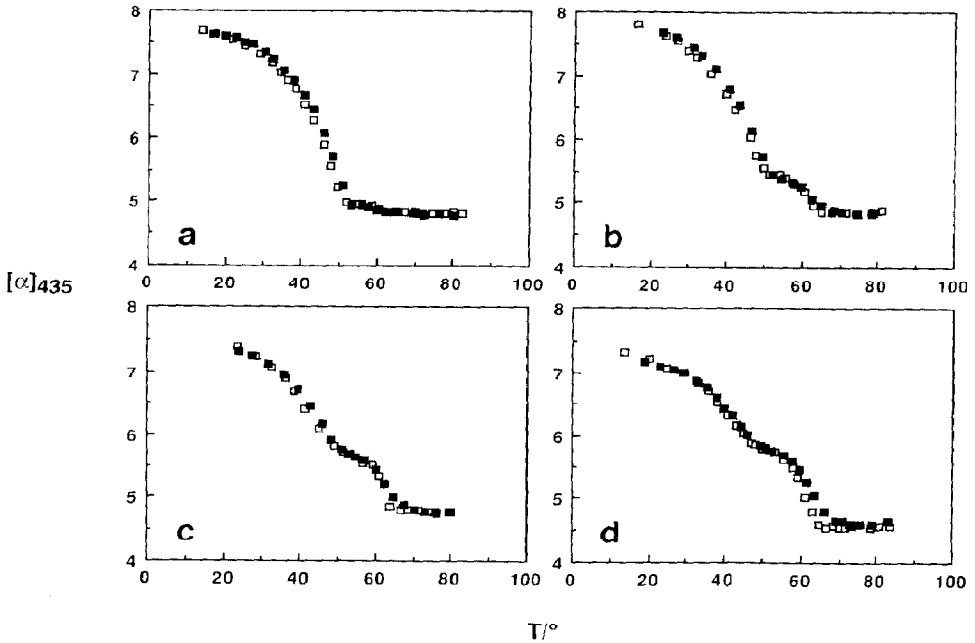

Fig. 4. $[\alpha]_{435}$ values for 1% mixtures of segmented rubidium iota- (from *E. spinosa*) and kappa-carrageenan (from *E. cottonii*), in 0.1M RbCl, as functions of temperature. Open and filled symbols refer to cooling and heating runs, respectively. The weight fractions of rubidium kappa-carrageenan were (a) 0, (b) 6, (c) 16, and (d) 22%.

Mixed carrageenan systems. — Under suitable conditions, iota- and kappa-carrageenans in mixtures undergo separate, thermally induced, conformational transitions[27]. Segmented rubidium carrageenans (see Experimental) as 1% solutions in 0.1M rubidium chloride were chosen for this study for the following reasons. Segments were used since intact carrageenans produce hazier and sometimes birefringent systems. The large excess of salt ensured that the activity of the rubidium ion was only weakly dependent on the kappa/iota ratio, the carrageenan/salt ratio gave rise to cation-specific n.m.r. effects of a suitable magnitude, and the conformational transitions of kappa- and iota-carrageenan molecules in mixed samples were well separated. Fig. 4 shows the temperature dependence of the optical rotation of various mixtures of carrageenan with up to 22% kappa-carrageenan. At higher percentages, the optical rotation readings became erratic, which is characteristic of concentrated systems of kappa-carrageenan[5,27].

Fig. 4. shows that the transition curves for iota-carrageenan were reversible even in mixed samples and the onset of helix formation occurred at 50 ±2° in each sample. Similar conclusions hold for the transition of kappa-carrageenan except that there was hysteresis, indicative of aggregation, which became more pronounced as the content of kappa-carrageenan increased. Also for kappa-carrageenan, the temperature of the onset of helix formation on cooling (64 ±2°) was independent of the composition of the sample and equal to the transition temperature observed for a sample prepared from only kappa-carrageenan (data not shown). This insensitivity of the coil-to-helix transitions to the relative proportions of iota- or kappa-carrageenan (at constant rubidium activity) strongly supports the conclusion[27] that the two molecular transitions occur independently in the mixture, and that no mixed aggregates are formed.

The temperature of final melting of all aggregated helices of kappa-carrageenan on heating seems to depend on the content of kappa-carrageenan. The aggregation of kappa-carrageenan is a non-equilibrium process and may proceed to different extents depending on the concentration of kappa-carrageenan and the amount of iota-carrageenan present.

Figs. 5 and 6 show the temperature dependence of the rubidium relaxation in a mixture containing iota-carrageenan and 22% of kappa-carrageenan, and a system containing iota-carrageenan alone. The apparent intensities and the two components of the transverse relaxation, R_2^+ and R_2^-, (see Experimental) are plotted as functions of temperature. All data refer to heating experiments. The temperatures at which the two types of carrageenan are completely converted into the coil state, as deduced from Fig. 4, are also indicated. As the hysteresis increases with increasing content of kappa-carrageenan, the final melting temperature of kappa-carrageenan is estimated to be 70° and 64° in Figs. 5 and 6, respectively.

The data in Fig. 5 show that the large effects on the rubidium relaxation in the mixed sample correlate with the transition step of the kappa-carrageenan, as all measured parameters, R_2^-,R_2^+, and the intensity of the rubidium signal, changed markedly over the temperature interval of the kappa-carrageenan transition. At

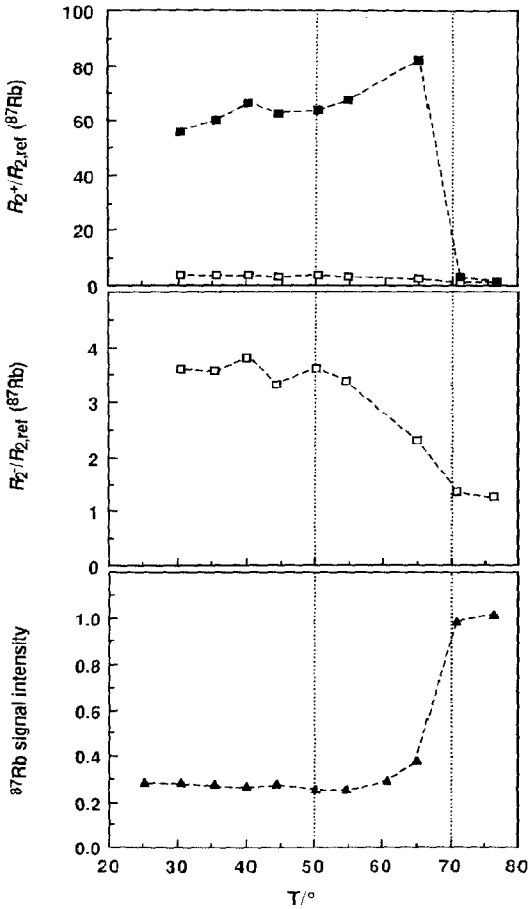

Fig. 5. Line-shaped [normalized R_2^+ (■) and R_2^- (□)] and relative signal intensity (▲) of the $^{87}Rb^+$ signal in a 1% rubidium carrageenan mixture (22% kappa-carrageenan) in 0.1M RbCl as a function of temperature. Vertical dotted lines indicate the temperatures where the helix-to-coil transitions of the iota (left) and kappa (right) fractions are complete (see text).

higher temperatures, the two transverse relaxation rates merged and there was a single lorentzian peak of full intensity. Conversely, at temperatures below that of the kappa-carrageenan transition, the intensities and the normalized relaxation rates reached constant values and were insensitive to the conformational transition of the iota-carrageenan which occurred at <50°. Thus, all the parameters measured, including the slow transverse relaxation rate R_2^-, were more sensitive to the transition of kappa-carrageenan than to the transition of iota-carrageenan.

The rubidium relaxation conformed to the same picture also for the sample containing no added kappa-carrageenan, as seen in Fig. 6. Although the effects on the rubidium relaxation were smaller than those of Fig. 5, they were dramatic and

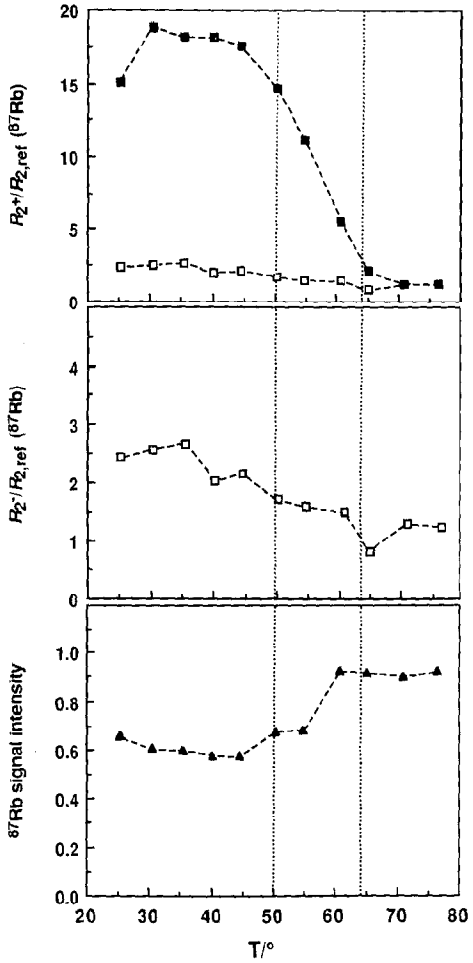

Fig. 6. As for Fig. 5, but for 1% rubidium iota-carrageenan.

occurred over the temperature interval characteristic of the transition of kappa-carrageenan under these conditions. Only for R_2^- was there a slight variation at <50°, reflecting the different intercharge separations and, hence, non-specific ion-binding properties of the iota-carrageenan coil and helix conformations. Thus, the small fraction of residues of kappa-carrageenan structure present in the sample of iota-carrageenan was responsible for the ion binding seen in rubidium n.m.r. Furthermore, this kappa-carrageenan fraction clearly gave rise to a separate, co-operative order–disorder transition which occurred in the temperature interval characteristic of "pure" kappa-carrageenan. This transition corresponds to the small step at >50° in the optical rotation curve for the sample containing no added kappa-carrageenan (Fig. 4). This implies that at least a portion of the kappa-carrageenan impurities in iota-carrageenan are located in long blocks of uniform

primary structure rather than as isolated desulfated units. Whether these regular kappa-carrageenan structures occurred as pure polymers of kappa-structure or as blocks in copolymers of hybrid kappa/iota-structure (or both) is not clear, but their presence would be expected to have consequences for the rheological properties and cation specificity of natural iota-carrageenan.

Concluding remark. — Although a quantitative interpretation of the n.m.r. relaxation of ions in polysaccharide gels is difficult, its sensitivity to ion binding may be utilized to obtain information on the occurrence and the transitions of structural impurities, which may be difficult to extract by other methods. In cases where these structures may act as potent cross-linkers, like kappa-carrageenan, this information should be valuable for the interpretation of the rheological behavior of the systems.

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REFERENCES

1 T. PAINTER, in G. O. ASPINALL (Ed), *The Polysaccharides,* Vol. 2, Academic Press, New York, 1983, pp. 195–285.
2 D. A. REES, E. R. MORRIS, D. THOM, AND J. K. MADDEN, in G. O. ASPINALL (Ed.), *The Polysaccharides,* Vol. 1, Academic Press, New York, 1983, pp. 195–290.
3 T. A. J. PAYENS AND T. SNOEREN, *J. Electroanal. Chem.*, 37 (1972) 291–296.
4 M. RINAUDO, A. KARIMIAN, AND M. MILAS, *Biopolymers*, 18 (1979) 1673–1683.
5 C. ROCHAS AND M. RINAUDO, *Biopolymers*, 19 (1980) 1675–1687.
6 C. ROCHAS AND M. RINAUDO, *Biopolymers*, 23 (1984) 735–745.
7 C. ROCHAS, *Food Hydrocolloids*, 1 (1987) 215–225.
8 E. R. MORRIS, D. A. REES, AND G. ROBINSON, *J. Mol. Biol.*, 138 (1980) 349–362.
9 I. T. NORTON, D. M. GOODALL, E. R. MORRIS, AND D. A. REES, *J. Chem. Soc., Faraday Trans. 1*, 79 (1983) 2501–2515.
10 K. R. J. AUSTEN, D. M. GOODALL, AND I. T. NORTON, *Biopolymers*, 27 (1988) 139–155.
11 I. T. NORTON, E. R. MORRIS, AND D. A. REES, *Carbohydr. Res.*, 134 (1984) 89–101.
12 V. J. MORRIS AND G. R. CHILVERS, *J. Sci. Food Agric.*, 32 (1981) 1235–1241.
13 V. J. MORRIS AND G. R. CHILVERS, *Carbohydr. Polym.*, 3 (1983) 129–141.
14 P. S. BELTON, G. R. CHILVERS, V. J. MORRIS, AND S. F. TANNER, *Int. J. Biol. Macromol.*, 6 (1984) 303–308.
15 O. SMIDSRØD AND H. GRASDALEN, *Hydrobiologia*, 116/117 (1984) 19–28.
16 M. WATASE AND K. NISHINARI, *J. Texture Stud.*, 12 (1981) 427–445.
17 M. WATASE AND K. NISHINARI, *Rheol. Acta*, 21 (1982) 318–324.
18 M. WATASE AND K. NISHINARI, *Colloid Polym. Sci.*, 260 (1982) 971–975.
19 M. WATASE AND K. NISHINARI, *Colloid Polym. Sci.*, 263 (1985) 744–748.
20 S. NILSSON, L. PICULELL, AND B. JÖNSSON, *Macromolecules* (in press).
21 S. NILSSON AND L. PICULELL, *Macromolecules*, in press.
22 H. GRASDALEN AND O. SMIDSRØD, *Macromolecules*, 14 (1981) 229–231.
23 S. PAOLETTI, F. DELBEN, A. CESARO, AND H. GRASDALEN, *Macromolecules*, 18 (1985) 1834–1841.
24 P. S. BELTON, V. J. MORRIS, AND S. F. TANNER, *Macromolecules*, 19 (1986) 1618–1621.
25 P. S. BELTON, V. J. MORRIS, AND S. F. TANNER, *Int. J. Biol. Macromol.*, 7 (1985) 53–56.
26 I. T. NORTON, D. M. GOODALL, E. R. MORRIS, AND D. A. REES, *J. Chem. Soc., Faraday Trans. 1*, 79 (1983) 2475–2488.
27 L. PICULELL, C. HÄKANSSON, AND S. NILSSON, *Int. J. Biol. Macromol.*, 9 (1987) 297–301.

28 L. PICULELL AND S. NILSSON, *J. Phys. Chem.*, in press.
29 C. BELLION, G. K. HAMER, AND W. YAPHE, *Proc. Int. Seaweed Symp.*, 10 (1981) 379–384.
30 T. A. BRYCE, A. H. CLARK, D. A. REES, AND D. S. REID, *Eur. J. Biochem.*, 122 (1982) 63–69.
31 P. S. HUBBARD, *J. Chem. Phys.*, 53 (1970) 985–987.
32 L. G. WERBELOW, *J. Chem. Phys.*, 70 (1979) 5381–5383.
33 B. HALLE, *Mol. Phys.*, 53 (1984) 1427–1461.
34 B. HALLE, *Mol. Phys.*, 60 (1987) 319–370.
35 S. FORSÉN AND B. LINDMAN, *Methods Biochem. Anal.*, 27 (1981) 289–486.
36 B. LINDMAN, in P. LASZLO (Ed.), *NMR of Newly Accessible Nuclei*, Vol. 1, Academic Press, New York, 1983, pp. 193–231.
37 J. R. ZIMMERMAN AND W. E. BRITTIN, *J. Phys. Chem.*, 61 (1957) 1328–1333.
38 B. HALLE, H. WENNERSTRÖM, AND L. PICULELL, *J. Phys. Chem.*, 88 (1984) 2482–2494.
39 G. GUNNARSSON AND H. GUSTAVSSON, *J. Chem. Soc., Faraday Trans. 1*, 78 (1982) 2901–2910.
40 L. PICULELL, B. LINDMAN, AND R. EINARSSON, *Biopolymers*, 23 (1984) 1683–1699.
41 H. WENNERSTRÖM, B. LINDMAN, S. ENGSTRÖM, O. SÖDERMAN, G. LINDBLOM, AND G. J. T. TIDDY, *NATO Adv. Study Inst. Ser., Ser. C*, 61 (1980) 609–614.
42 J. ANDRASKO, *J. Magn. Reson.*, 16 (1974) 502–504.
43 O. SMIDSRØD AND H. GRASDALEN, *Hydrobiologia*, 116/117 (1984) 178–186.