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Molecular Organization of Kappa Carrageenan in Aqueous Solution

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SUMMARY

The interaction of K^+ with unsegmented kappa carrageenan in aqueous solution was studied by conductimetry, optical rotation and light scattering. By comparing the results from the different techniques it is possible to propose the existence of an equilibrium between molecules as single-helix and helical-dimer depending on the ionic concentration.

INTRODUCTION

Kappa-carrageenan is an algal polysaccharide based on a linear alternating sequence (Fig. 1) of 3-linked β-D-galactopyranose-4-sulfate and 4-linked, 3,6-anhydro-α-D-galactopyranose. The ordered conformation of this polymer is well known. Goring and Chepeswick (1955) suggested that some properties of carrageenan in solution could be related to the formation of a helix. Strong evidence for the presence of a helical conformation was presented by Rees and coworkers (Rees *et al.*, 1970; Rees, 1977; Norton *et al.*, 1979; Bryce *et al.*, 1982) and further proof that the

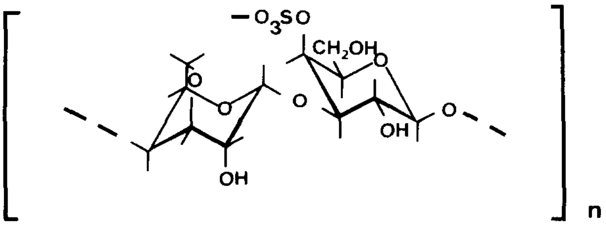

Fig. 1. Primary structure of kappa carrageenan.

ordered polysaccharide chains contained double helical entities came later from stopped-flow polarimetric measurements. (Norton *et al.*, 1983). In contrast, Smidsrød proposed a single-helix model to explain some of his experimental results (Smidsrød, 1980). We have suggested the concept of helical-dimers on the grounds of thermodynamic experiments and results from conductimetry, potentiometry and calorimetry (Rochas and Rinaudo, 1980; Rochas and Rinaudo, 1982; Rochas and Mazet, 1984). The ordered conformation, single helix or helical dimer, is obtained by comparing the experimental data obtained from these techniques and the results obtained from the polyelectrolyte theory of Manning (Manning, 1969). In addition, we mentioned some of the possible errors that can arise when one is using a rough comparison between the calorimetric results and Manning's theory. In this regard, we showed that it was better to use a calorimetric technique in conjunction with another thermodynamic method (Rochas, 1985). These results led us to depict in a diagram the different domains of existence of the disordered chains and helical dimers (region I and regions III and IV respectively, Fig. 2). When the ionic concentration C_T is higher than C^* the helical dimers are aggregated in a gel phase (region III) and the conformational transition shows hysteresis. In contrast when $C_T < C^*$ the helical-dimers are free and the conformational transition occurs without

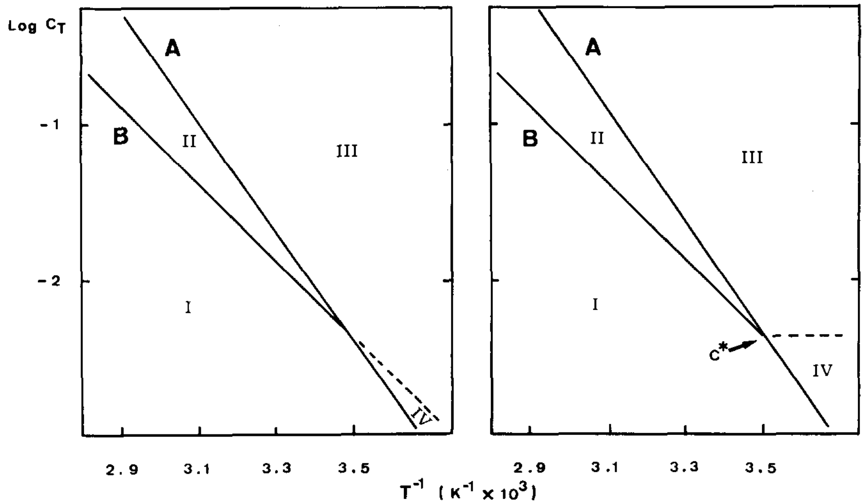

Fig. 2. Phase diagram of kappa carrageenan. C_T represents the total potassium concentration. The left diagram was presented by Paoletti *et al.* and the right diagram by Rochas *et al.* The different regions are identified in the text.

hysteresis (region IV, I). It is important to stress that region II (hysteresis) in the graph does not correspond to the domain of existence of a particular conformation, but rather to the overlap of two graphs corresponding to the hysteresis. One graph is delimited by line A and represents the conformational change during the cooling process whereas the other, delimited by the line B, corresponds to the conformational change during the heating process.

Recently Paoletti *et al.* (Paoletti *et al.*, 1984, 1985) described a model for the ordered conformation of kappa carrageenan in which the molecules would exist as a combination of single helices and helical-dimers that would fit their calorimetric results. They interpreted our diagram (Rochas and Rinaudo, 1980; Rinaudo and Rochas, 1981; Rochas and Rinaudo, 1984) by claiming that, in contrast to our proposal, region III (Fig. 2) corresponds to the domain of existence of single helices and helical dimers. They also extended line B to obtain a novel region, region IV (Fig. 2) where disordered chains and single helices would be present. The existence of this region is not based on experimental data but is merely an assumption. In our diagram, region IV can be considered as a particularity of region III where the transition occurs without hysteresis. In addition, they proposed the existence of helical dimers and disordered chains in region II (hysteresis). In contrast to the Italian school who obtained their results using isothermal calorimetry, we give another view of the association of the kappa-carrageenan ordered chains using isoionic techniques.

MATERIALS AND METHODS

The kappa-carrageenan sample (described in this paper) was obtained from Sigma and was used after purification (Rochas and Rinaudo, 1980). The specific rotation of the coil form at 25°C and recorded at 365 nm was 180°. Similar results were obtained from a sample of kappa-carrageenan supplied by Ceca (Carentan, France).

The optical rotation was measured at 365 nm on a Perkin-Elmer 241 polarimeter using quartz cells of 10, 20, or 100 mm path length. The conductivities were measured at 250 Hz on a conductivity bridge (from Tacussel, France) with a micro-cell (Tacussel CM 08). The conductivity and optical cells were thermostated and the temperature controlled by a thermocouple.

The refractive index increments (*dn*/*dc*) were measured on a differential refractometer Iota (Jobin Yvon, France) calibrated against potassium chloride solutions. For light scattering experiments the solutions were filtrated on membranes 0·22 μm (Sartorius, France). The average molecular weight was established from a low angle light scattering KMX6 system (Chromatix, USA), at room temperature. The change in molecular weight due to the conformational change (see Table 2) was observed by Size Exclusion Chromatography. The elution was performed at 25°C on B 804 and 805 columns (Shodex, Japan) at a flow rate of 0·5 ml min^{-1}. The small tube placed between the end of the column and the thermostated cell of the KMX 6 system was also thermostated. Due to the thermostated volume between the end of the column and the light scattering cell, and to the flow rate, the residence time of the eluent at a controlled temperature was estimated to be 2–2·5 min. From optical rotations experiments, this time was sufficient for a complete transition to occur.

Due to the low ionic content of the eluent the virial coefficient cannot be neglected during the SEC process. To overcome this problem, different amounts of polymer were chromatographed and the light scattering signal used to determine the molecular weight was obtained by extrapolation of the experimental signals to zero polymer concentration.

RESULTS AND DISCUSSION

The degree of molecular order of carrageenan is readily obtained by optical rotation (Fig. 3). A maximum of order is reached when the ratio [α] all helix/[α] all coil is 1·56 and cannot be changed even though the ionic concentration or the time of observation are increased. Similar ratios were reported in the literature (Bryce *et al.*, 1982). In a manner exactly similar to that observed by optical rotation the degree of molecular order at a temperature T is obtained by the conductivity $\varkappa$ observed at a temperature T from the ratio (Fig. 3).

$$\frac{\varkappa_{\text{coil}} - \varkappa_{\text{observed}}}{\varkappa_{\text{coil}} - \varkappa_{\text{helix}}}$$

If the total ionic concentration (taking into account the potassium from the polymer and/or the added electrolyte according to Rochas and Rinaudo, 1982) is very low, the conformational transition induced by temperature is time dependent (Fig. 4) and a maximum of order cannot be reached if the cooling rate is fast. A reduction of this rate or an increase in the observation time at low temperature would be required to observed a maximum order.

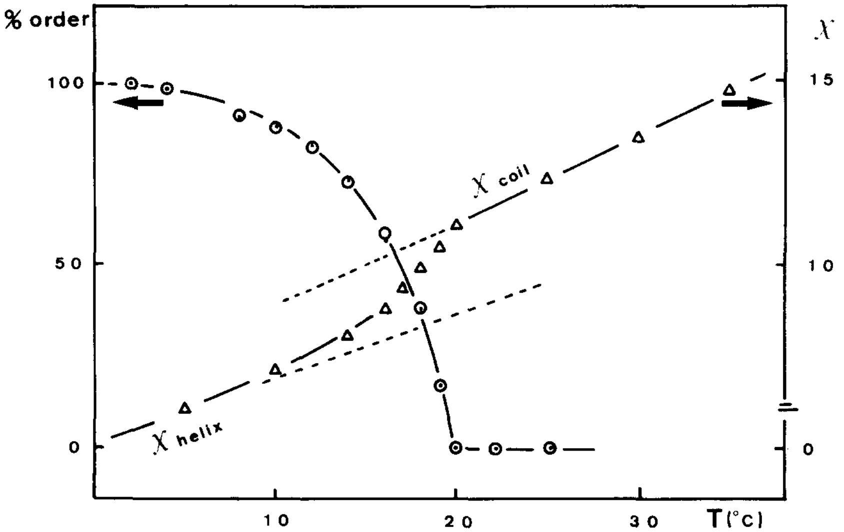

Fig. 3. Percentage of order of kappa carrageenan (potassium form, concentration 5·04 g^{-1} litre) deduced from optical rotation (○) and conductivity measurements, (●). The figure also shows measured values for the conductivity, (△), expressed in mS.

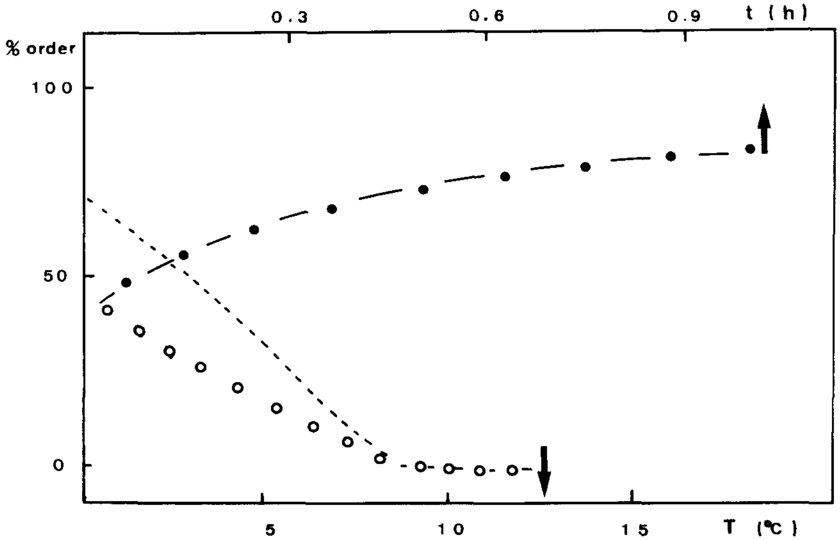

Fig. 4. Percentage of order of kappa carrageenan (potassium form, 1·4 g^{-1} litre) observed in different conditions and determined by conductivity or polarimetry: (○) versus temperature, cooling rate 25°C h^{-1}, (–) versus temperature, cooling rate 5°C h^{-1}; (●) versus time recorded immediately after the A run.

Nevertheless, the conductivity and optical rotation curves are exactly superimposed (Figs 3 and 4), whatever the cooling rate, observation time, concentration of the polymer or concentration of salt mixed with the polymer. With kappa-carrageenan, a conformational transition is always detected by a variation in optical rotation and conductivity. The latter reflects the change in the charge density associated with any total or partial dimerization that may be occurring during the helix-coil transition. The conductivities of the coil and helix may be written:

$$\varkappa_c = Kf_c\Lambda_p \tag{1}$$

$$\varkappa_h = Kf_h\Lambda_p \tag{2}$$

where K is a constant including the polymer concentration, the solvent and units adopted; f is the coefficient of conductivity (Manning, 1974) and Λ_p the equivalent conductivity of the polyelectrolyte. Assuming Λ_p is constant with the conformation, $(\varkappa_c/\varkappa_h)=(f_c/f_h)$. According to Manning (Manning, 1969, 1974):

$$f = 1 - \frac{0{\cdot}55\lambda^2}{\lambda + 3{\cdot}14} \leqslant 1 \tag{3}$$

$$f = \frac{0{\cdot}87}{\lambda} \geqslant 1 \tag{4}$$

where λ is the charge parameter in the polyelectrolyte theory. From the structural data of kappa-carrageenan, different charge parameters can be computed (Rochas and Rinaudo, 1980):

$$\lambda_{\text{coil}} = 0{\cdot}68$$

$$\lambda_{\substack{\text{single}\\ \text{helix}}} = 1{\cdot}65/2$$

$$\lambda_{\substack{\text{helical}\\ \text{dimer}}} = 1{\cdot}65$$

Due to the transition and polyelectrolyte properties of the polymer, different changes in conductivity $(\Delta\varkappa = \varkappa_{\text{coil}} - \varkappa_{\text{helical form}})$ can be derived from eqns 3 and 4 for the two possible conformational changes: coil-single helix and coil-helical dimer. The conductivity between the coil state and the ordered conformation for different concentrations of polymer and salt versus the melting temperature T_m of the investigated solution is reported in Fig. 5. Two main characteristics arise from Fig. 5. First, the points obtained for polymer solutions or mixture of polymer

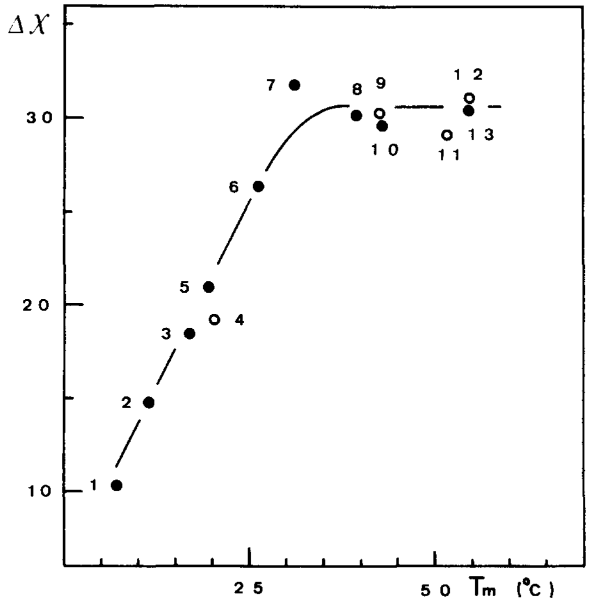

Fig. 5. Increase in conductivity (mS) for solution of potassium form of kappa carrageenan with or without KCl added. From point 1 to point 13 the solvent is water and the concentrations are respectively: 1·63, 2·52, 5·04, 2·52, 6·73, 10·01, 19·6, 40·2, 10·5, 52·7, 10·5, 20·1 and 57·8 g litre^{-1}. Points 4, 9, 11, 12 are solutions prepared with 0·0047, 0·05, 0·1 and 0·1 M KCl, respectively.

and salt are on the same curve. This fact demonstrates, as previously mentioned (Rochas and Rinaudo, 1980), that the free counterions coming from the polymer and from the salt added are equivalent. Secondly, for T_m temperatures above 35°C the change of conductivity is constant, but for temperatures lower than 35°C this variation decreases from 30 to 10 mS eq^{-1}. The relative increase in conductivity $\Delta x/x$ (x coil $-$ x ordered/x ordered form) of polymer without salt is depicted on Fig. 6 where we obtain trends similar to those of Fig. 5. From 35 to 60°C $\Delta x/x$ is constant ($0{\cdot}80 \pm 0{\cdot}05$); from 35 to 7°C, $\Delta x/x$ decreases from 0·80 to 0·19 for a concentration of 1·63 g litre^{-1} of kappa-carrageenan in the potassium form and for concentrations lower than 1·63 g litre^{-1} the chains are not completely ordered.

From the relations of eqns 3 and 4 and taking into account the three possible values of the chain parameter, we can assume a $\Delta x/x$ value of 0·03 for the single helix-coil step or a value of 0·75 for the helical dimer-coil step. Consequently, from Figs 5 and 6 we can conclude that, if the conformational melting temperature of a solution is above 35°C, the conformational transition goes from a helical dimer to a coil. On the

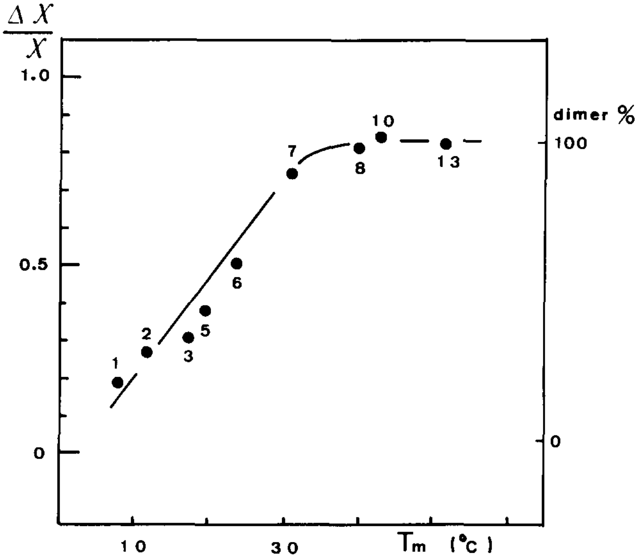

Fig. 6. Relative increase ($\Delta x/x$ ordered) in conductivity of a solution of the potassium form of kappa carrageenan versus temperature. The right axis represents the proportion of single helix and helical dimers. Points as in Fig. 5.

other hand, if the conformational melting temperature of a solution is lower than 35°C, the coexistence of single helices and helical dimers can explain the drastic decrease of $\Delta x/x$ with temperature (Fig. 6).

Assuming a logarithmic relation between the total ionic concentration and the melting temperature (Rochas and Rinaudo, 1980), we can change the representation of Fig. 6 and plot $\Delta x/x$ against the total ionic concentration (Fig. 7). For this rough approximation, we computed the ionic contribution of the polymer, as previously published, and assumed, as it is generally done, that 50% of coil and 50% of the ordered forms are present at T_m. According to Fig. 6, the ordered form is composed of a certain proportion of single helix and helical dimer, assuming that only single helices are formed below 8°C and only helical dimers are formed at temperature above 35°C.

In Fig. 7 $\Delta x/x$ is plotted against the ionic content. When the ionic content is higher than 3×10^{-2} M, helical dimers are observed. When the ionic content is lower than this limit, the proportion of helical-dimers decreases and the single-helix content increases. Preliminary results on low molecular weight kappa-carrageenan are similar. For partially desulphated kappa carrageenan the Δx value differs slightly due to the change in the charge parameters λ_c and λ_h in connection with eqns 1–4. More generally, whatever the molecular weight or charge density of the

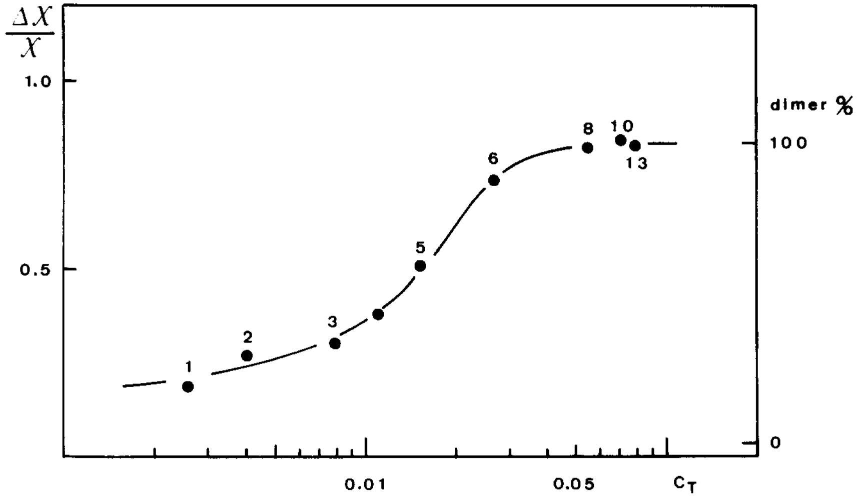

Fig. 7. Relative increase ($\Delta x/x$ ordered) in conductivity of solution of potassium form of kappa carrageenan versus ionic content C_T. The right axis represents the proportion of single helix and helical dimers. Points as in Fig. 5.

sample, if the ionic content is zero, only single-helices exist but it is a virtual point. This point is virtual because the polymer contribute to the ionic content and consequently a zero ionic content is not possible. Consequently the transition on Fig. 7 can also be described from molecular weight measurements.

We know that the ordered chains are aggregated when the ionic content C is higher than C^* (Rochas and Rinaudo, 1980). If C is lower than C^* the chains are free and it is possible to perform some light scattering experiments on the coil and ordered forms. From the diagram (Fig. 2), an ionic content of 5×10^{-3} complies with this condition. In addition at 25 and 7°C the chains are disordered and ordered, respectively. However, in order to perform light scattering experiments, several conditions need to be respected. The concentration of the polymer in solution has to be lower than the overlapping concentration C_0. (Hara and Nakajima, 1980*a*, 1980*b*) and the solution has to be in a Donnan equilibrium.

The overlapping concentration C_0 can be approximated by the relationship below (Graessley, 1980)

$$C_0 = \frac{0{\cdot}77}{[\eta]}$$

where $[\eta]$ is the intrinsic viscosity.

From Table 1, one condition of our experiments appears: the polymer concentration has to be lower than 0·47 g litre^{-1} for an ionic content of 5×10^{-3} M. The values of C_0 from the viscosity (Table 1) are in agreement with the light scattering experiment (Fig. 8). During size exclusion chromatography (SEC) the polymer is eluated at a concentration lower than C_0 (see materials and methods for conditions).

TABLE 1
Overlapping Concentration of Kappa Carrageenan in Various Conditions and Weight Average Molecular Weight

	Solvent at 25°C		
	KCl 5×10^{-3}	*NaCl 5×10^{-3}*	*NaCl 1×10^{-1}*
$[\eta]$ (ml g^{-1})	1650	1630	700
C_0 (g litre^{-1})	0·47	0·48	1·10
M_W	$5{\cdot}5 \times 10^5$	$5{\cdot}5 \times 10^5$	$5{\cdot}5 \times 10^5$

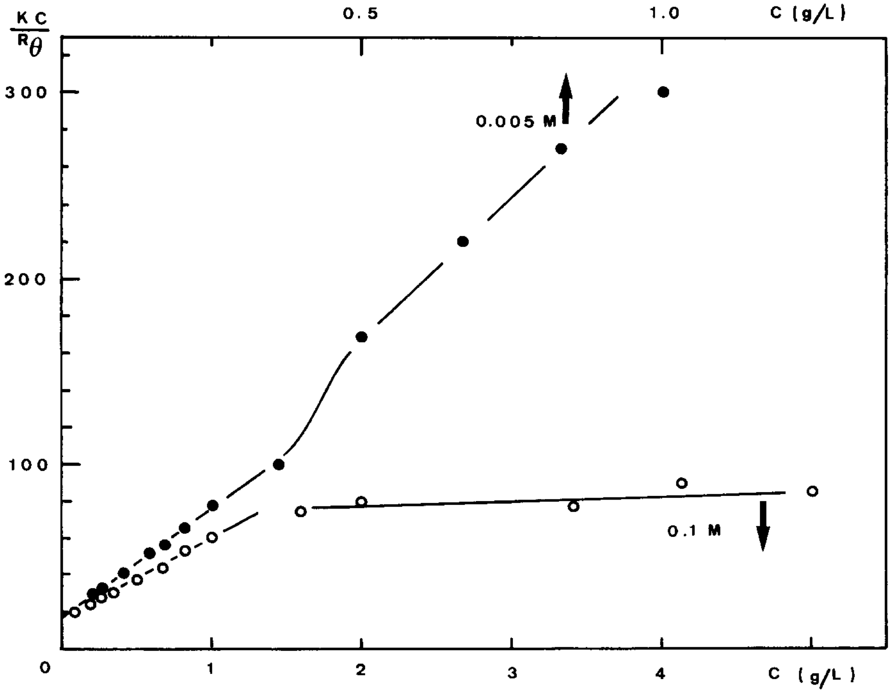

Fig. 8. Low-angle light scattering data from an analysis of kappa carrageenan: (●) solution in 5×10^{-3} M NaCl (○) solution in 0·1 M NaCl, KC/Rθ is expressed in arbitrary units.

The final condition for the light scattering of polyelectrolytes is that it is necessary to work with solutions of chemical potential similar to that of the solvent (Donnan equilibrium). This equilibrium is realized for a polymer solution that has been chromatographed by size exclusion chromatography (Rochas *et al.*, 1980) (see materials and methods for conditions). Consequently SEC respects both the conditions required for the experiment: polymer concentration lower than C_0 and use of solution after a Donnan equilibrium. From Fig. 7, an ionic concentration of 5×10^{-3} M KCl gives a value of $\Delta\chi/\chi$ of 0·35. Therefore, we can expect a molecular weight increase of 35% which means that 35% of the chains are helical-dimers and 65% are single-helices. The experimental value is in agreement with this variation and is not an artefact due to the temperature, because in the presence of NaCl no change in molecular weight is detected between 25 and 2°C (Table 2).

With these results, a slight modification of our diagram in Fig. 2 can be made. Region I, II, III are identical to the diagram previously published: I, coil region; II, overlap between 2 graphs corresponding to the hysteresis (see before); III, aggregated helical chains; IV, free helical chains.

In addition, from these new results, we can postulate that for the ordered chain the ionic content C_T directs the equilibrium between single helices and helical-dimers formation.

$$\begin{array}{lcl} \text{single helix} & \overset{C_T'}{\rightleftharpoons} & \text{helical dimer} \\ 100\% \text{ at } C_T = 0 & & 100\% \text{ for } C_T > 3 \times 10^{-2}\text{ M} \end{array}$$

Due to the same trends for optical rotation versus temperature, and conductivity versus temperatures (Fig. 3), we can conclude that the helical dimers do not appear after the single helices if the temperature

TABLE 2

Ratio of the Weight Average Molecular Weight Determined by SEC Experiment in Various Conditions to the Weight Average Molecular Weight Determined by the Static Experiment (Fig. 8)

Temperature (°C)	25	25	2	2
Salt 5×10^{-3} M	NaCl	KCl	NaCl	KCl
State	Coil	Coil	Coil	Ordered
dn/dc	0·126	0·126	0·126	0·123
Ratio ± 0·06	1·0	1·0	1·0	1·38
$\overline{M_w}$	$5{\cdot}5 \times 10^5$	$5{\cdot}5 \times 10^5$	$5{\cdot}5 \times 10^5$	$7{\cdot}6 \times 10^5$

is decreased below the temperature where 100% of order is found. The balance single helix–helical dimer is only driven by the ionic content.

A single-helix conformation of kappa-carrageenan is possible only when the ionic content is low. However, such conditions are never met with potassium because (Fig. 7), the transition is incomplete or does not exist for a very low potassium content. A coexistence of single helices and helical-dimers is postulated here for kappa-carageenan at low ionic content in water. It has already been proposed that a single-helix conformation can exist in organic solvents (Rochas and Rinaudo, 1982). In addition, preliminary conductimetric results show that iota-carrageenan is a helical dimer when the ionic concentration is high. However, when the salt concentration is low it resembles a combination of a single and helical dimer. A single-helix conformation has also been proposed for agarose under some conditions (Bouffar-Roupe *et al.*). Consequently, it is possible to postulate that a single helix conformation is an intermediate step during the transition of the galactan family. Such conformation could appear before, or with, helical dimer formation, depending on the temperature and on the solvent composition.

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