Communications to the Editor

Molecularity of the Salt-Induced Conformational Transition of ι-Carrageenan

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Introduction. An important element in the structure/property relations in carrageenans (linear sulfated galactans from red algae) is an ion- and temperature-induced transition from a disordered to an ordered conformation.[1-3] However, since long a controversy exists as to the nature of the basic ordered conformation in aqueous solutions, i.e., the one which can be assumed to be a prerequisite for any further chain interaction, eventually leading to gel formation.

A coaxially intertwined double helix was postulated on the basis of X-ray fiber diffractograms and stopped-flow kinetic data by Rees and co-workers.[4-8] On the other hand, Smidsrød and co-workers have proposed a model envisaging a single-stranded disordered to single-stranded ordered conformation, for both κ- and ι-carrageenans.[2,9-11]

As far as κ-carrageenan is concerned, an extensive experimental study, mainly based on light scattering, has shown that at least for aqueous NaI solutions the onset of such transition can take place at low polymer concentrations without any significant change of the polymer molar mass.[12] Only a few papers based on light scattering have been published so far for the more highly charged ι-carrageenan[1,13-16] (which bears two sulfate charges per disaccharide repeating unit): they point to an increase of the $\langle M\rangle_w$ upon decreasing temperature and/or increasing salt concentration.

There are intrinsic difficulties arising in the determination of the molecular weight for systems which may be suspected to associate: (i) the choice of the polymer concentration range, which is often not wide enough; (ii) complications connected with the double extrapolation procedure (Zimm plots) and hence the need to check the convergency of M_w data obtained from independent static wide-angle laser light scattering (WALLS) and low-angle laser light scattering (LALLS) experiments; (iii) the assessment of pitfalls related with the preparation of the solutions and careful monitoring of eventually occurring time effects.

Experimental Section. ι-Carrageenan is a commercial sample from Sigma Chemical Co., Type V, Lot 27 F0373; κ-type impurities are less than ca. 5% (as determined by NMR), of which full details will be given elsewhere.

The following procedure was used for the preparation of solutions: starting from an aqueous carrageenan solution of known concentration, a fixed amount of a salt solution was added, followed by filling up the volumetric flask with pure water to the graduation mark to realize the desired salt molarity. The solutions were stored for 24 h at room temperature. Concentrations of ι-carrageenan used for the light scattering experiments were chosen in the range between 0.05 (sometimes even lower) and 0.4 g/L (sometimes higher).

Static light scattering at 25 and 45 °C was measured at angles between 30° and 100° using an Amtec MM 1000 photometer[12] in conjunction with a He-Ne laser yielding a polarized primary beam of wavelength 633 nm. The Rayleigh ratio for benzene,[17] R_b, at 633 nm was taken as 11.84×10^{-6} cm^{-1}. This value was used at room temperature as well as for the measurements obtained at 45 °C. All solutions were filtered for several minutes through Millipore filters (ϕ 0.22 μm) using a closed circuit involving a filter holder and a peristaltic pump. The cylindrical cell, containing the solution, was immersed into a thermostatically controlled toluene bath. The data were further analyzed according to the normal Zimm procedure.

A Chromatix KMX-6 was used at an angle less than 6° and $\lambda = 633$ nm for the LALLS. The carrageenan solutions, previously filtered according to the arrangement of the static angular dependent light scattering, were pumped continuously through the measuring cell after passing a Millipore filter of 0.22 μm.

Refractive index increment determinations were performed in conditions appropriate for light scattering experiments of ionic polysaccharides: the mean value obtained was 0.151 ± 0.09 mL/g which we used for all temperatures.[12] For all solutions studied the results of WALLS extrapolated to angle zero were within experimental accuracy in very good agreement with those obtained by LALLS.

Results and Discussion. Light scattering experiments have been performed on Na$^+$ ι-carrageenan samples dissolved in aqueous solutions of NaCl or NaI. The latter electrolyte, in contrast to the former one, is known to promote the stability of the molecularly disperse solutions of carrageenans, even in the presence of the ordered conformation.[2] Experiments were carried out at both 25 and 45 °C, the latter being assumed on the basis of optical activity data to be a temperature where the disordered state of the polymer prevails, over the whole salt concentration range explored.

At the start of the project the authors failed to obtain consistent and reproducible light scattering results under conditions where for κ-carrageenan normal polyelectrolyte behavior is observed. A time-dependent aggregation has been detected, which is the more pronounced, the higher the carrageenan concentration and the molarity of the salt solution. The light scattering of a freshly prepared Na$^+$ ι-carrageenan solution

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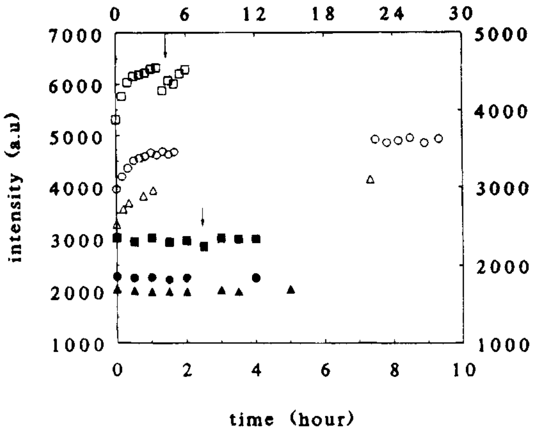

Figure 1. Light scattering intensity at angle 50° as a function of time of 0.37 g/L of Na$^+$ ι-carrageenan in (○) 0.08 M NaCl, (△) 0.08 M NaI, (●) 0.03 M NaCl, (▲) 0.03 M NaI, (□) 0.09 M NaCl. Right y-scale: (■) 0.09 g/L of Na$^+$ ι-carrageenan in 0.09 M NaCl. The arrow indicates the time of filtration. The three upper curves correspond to the upper x-scale; the three lower curves correspond to the lower x-scale.

(0.37 g/L) was measured as a function of time in 0.08 and 0.03 M salt solution (NaCl and NaI) as shown in Figure 1.

One observes a rapid increase of the light scattering intensity during the first 2 h following filtration and a more slow increase up to a constant value after approximately 5 h; the lower the carrageenan concentration (0.09 g/L), the less pronounced the effect. No increase in scattered intensity is observed after filtration in the cases where the polymer is dissolved in 0.03 M NaI or 0.03 M NaCl solutions. Such findings point to a nonequilibrium state of ι-carrageenan solutions at higher salt concentrations immediately after sample preparation. Another point of interest is the fact that in the case of ι-carrageenan, dissolved in a NaI solution, a less pronounced effect is observed. From the same figure it is obvious that when a solution is filtered again during the aggregation process, a decrease in the scattered intensity is observed. Dissociation of aggregates occurs also on heating the solutions up to 45 °C, as will be discussed subsequently. Hence, these findings create the potential to study aggregated solutions in an equilibrium state at room temperature as well as solutions free of aggregates at 45 °C. With respect to the order–disorder transition of ι-carrageenan, as detected by optical rotation, measurements at 45 °C correspond to the disordered state (coil) and those at room temperature to the ordered state (helix).

Taking into account the former findings, we report in Figure 2 the scattering function Kc/R_θ (for $\theta = 0$) obtained by LALLS measurements as a function of polymer concentration for three different values of the NaI concentration, at 25 °C. The highest value of the ionic strength (0.08 M) corresponds to the condition where the polysaccharide is in the ordered conformation, as evidenced by optical rotation measurements. Although at the highest ionic strength the concentration dependence of the scattering function shows a definite upward curvature, the extrapolated value to zero concentration coincides within experimental error with the values obtained from the extrapolation at the lower NaI concentrations and within experimental accuracy with the one derived at 45 °C in 0.08 M NaCl (Figure 3).

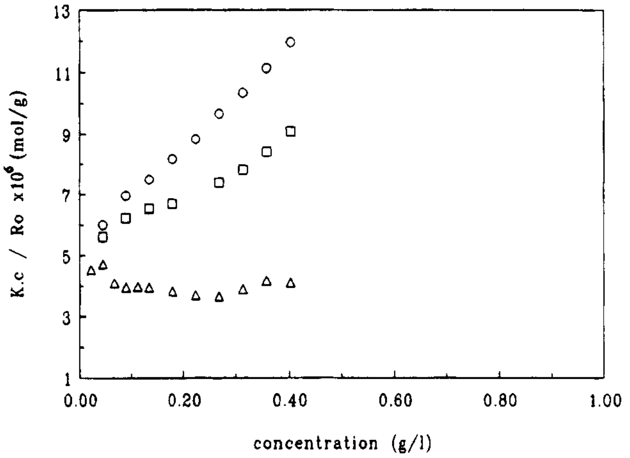

Figure 2. LALLS measurement of carrageenan in different NaI solutions: (○) in 0.03 M; (□) in 0.05 M; (△) in 0.08 M.

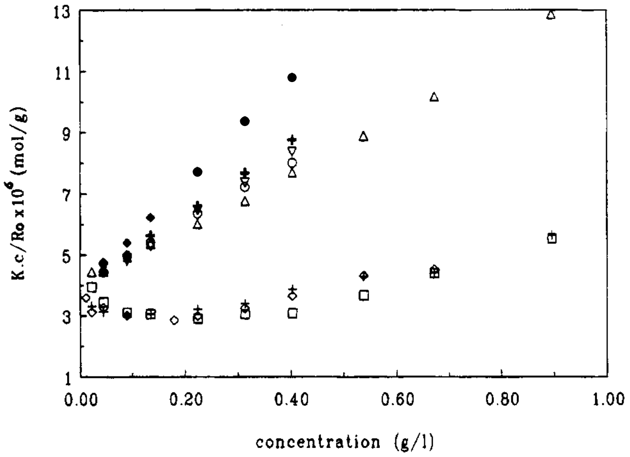

Figure 3. Reduced light scattering intensity at zero angle of ι-carrageenan in 0.08 M NaCl. At 25 °C: (◇) WALLS; (□) LALLS; (+) LALLS after cooling. At 45 °C for LALLS: (△) 0.08 M NaCl; (○) 0.06 M NaCl; (▽) 0.05 M NaCl; (+) 0.04 M NaCl; (◆) 0.02 M NaCl.

In Figure 3 similar WALLS and LALLS data have been reported for NaCl. Here again, at 0.08 M salt concentration and at 25 °C, an analogous curvature of the scattering functions has been observed. All data obtained in the different conditions extrapolate, within experimental accuracy, to the same values as in the NaI experiments. Optical rotation data at 0.08 M NaCl indicated the existence of conformational order but showed no dependence on the polymer concentration in the same range as that of the light scattering experiments.

From the whole of those observations, we cannot find any experimental evidence for dimerization accompanying the salt-induced conformational transition of ι-carrageenan, at least at very low concentrations.

If, instead of taking into account the curvature of the scattering function for the curve in 0.08 M NaCl and NaI, a linear extrapolation to zero concentration would have been performed from the data obtained in the high concentration range, it would have led to $\langle M\rangle_w$ which is higher than that of the disordered form. That value would be larger for NaCl than for NaI solutions.

Therefore, it can be concluded that the occurrence of the ordered state of ι-carrageenan at low polymer concentrations, in both NaI and NaCl, is not paralleled by a chain dimerization, thus ruling out the possibility of the existence of double-stranded ordered chains under those experimental conditions. Moreover, this observation is not limited to iodide solutions, for which peculiar effects have been claimed to take place for carrageenans.[2]

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