Macromolecules **1999,** *32,* 683−689

Equilibrium and Nonequilibrium Association Processes of κ-Carrageenan in Aqueous Salt Solutions

Karin Bongaerts and Harry Reynaers

Laboratory of Macromolecular Structural Chemistry, Department of Chemistry, K.U. Leuven, Celestijnenlaan 200F, Heverlee, Belgium

Flavio Zanetti

POLY-tech S.C.r.l., AREA Science Park, Padriciano 99, I-34012 Trieste, Italy

Sergio Paoletti*

Department of Biochemistry, Biophysics and Macromolecular Chemistry, University of Trieste, via L. Giorgieri 1, I-34127 Trieste, Italy

Received July 31, 1998; Revised Manuscript Received December 1, 1998

ABSTRACT: A large number of light-scattering data indicate that the sulfated polysaccharide κ-carrageenan undergoes an *intramolecular* conformational transition under suitable conditions from a semiflexible, disordered conformation to a rather rigid, helical one. By varying the parameters controlling the conformational phase diagram (i.e., by increasing the concentration of polymer or that of the supporting electrolyte, or both, or by decreasing temperature), a tertiary structure is manifested, through a progressive increase of the weight-average molar mass ($\bar{M}_w$) as determined by light scattering. This can be very well explained in terms of a reversible association of stretches of such intramolecular helical structures and quantitatively described by a model of "open association" of two or more chains. The association of κ-carrageenan was demonstrated to take place at around room temperature also in the presence of iodide ions, as long as their concentration is ≥0.20 M. In this way the apparent inconsistency of a number of published $\bar{M}_w$ results can be reconciled. Additional light-scattering results indicated that the demonstrated thermodynamic tendency to reversible interchain association may lead to an irreversible aggregation of polymer chains if an improper solution preparation procedure is used.

Introduction

Carrageenans are water-soluble, sulfated polysaccharides extracted from different species of marine red algae of the class Rhodophyceae. One of their basic properties is the ability to induce thickening or gelation of solutions. This process appears to be thermoreversible with or without hysteresis and has been the object of numerous studies.

Supramolecular structure formation in aqueous solutions of carrageenans is a complex process involving a temperature-induced disorder-to-order conformational transition and a chain-chain association superimposed over polyelectrolyte effects due to their ionic nature. Temperature, polymer concentration, ionic strength, and the nature of co- and counterions markedly influence and modulate these mechanisms in a complicated manner. In particular, the strong tendency to association contributed to hamper the achievement of a fast and universal consensus on the nature of the ordered conformation involved in those processes.

The behavior of κ-carrageenan is no exception to this rule. The primary structure of κ-carrageenan consists ideally of a regular alternation of α(1−3)-D-galactose-4-sulfate and β-(1−4)-3,6-*anhydro*-D-galactose, although other monosaccharides such as galactose, galactose-2-sulfate, galactose-6-sulfate, and galactose-2,6-disulfate may be present in minor amounts.

The scope of this paper, like that of the accompanying one,[1] is confined to a detailed analysis of molar mass determinations only, purposely combining a critical assessment of all significant data from the literature with new original data obtained to confirm previous results or to support the critical evaluation of others. This paper is devoted to the analysis of the intermolecular association aspects, both when they represent a true equilibrium state of the system and under conditions of metastability. Use will be made of the well-established formalism developed to analyze light-scattering data of associating polymeric systems.[2]

Experimental Section

Materials. The κ-carrageenan sample and the other chemicals were the same as described in the preceding paper.[1] Also, the low-angle laser light scattering (LALLS) setup and the measuring protocol was the same as previously described.[1]

The procedure for the preparation of solutions was the same as in ref 1, which follows strictly the protocol of ref 3. When explicitly desired, the protocol followed for the preparation of the polymer solution, in the presence of NaCl and NaI, is the one reported in ref 4.

The inverse reduced scattering intensity, $(KC_p)/R_\theta$, dependence on angle, θ, of the data of Figure 6b of ref 4 was analyzed. Visual inspection of the plot revealed a significant downward curvature. In fact, linear regression analysis gave a comparatively high value of the sum of the squares of the residuals, χ^2: $\chi^2 = 2.58 \times 10^{-14}$. A polynomial regression of the data points significantly reduced χ^2 to 2.87×10^{-15}. Consequently, the limiting values of $(KC_p)/R_\theta|_{\theta = 0°}$ from such a polynomial analysis were used for the calculation of $\bar{M}_w$ and of the second virial coefficient, A_2.

For the evaluation of the ionic strength, I, of the solutions of ref 8, the contribution of the polyelectrolyte counterions has been neglected for all values of the molarity of the supporting

---

* Corresponding author. Tel.: +39 040 676 36 92. Fax: +39 040 676 36 91. E-mail: paolese@bbcm.univ.trieste.it.

10.1021/ma981204r CCC: $18.00

electrolyte KCl, because the polymer concentration, C_p, was always much smaller than [KCl].

The Open Association Model of Elias. For carrageenan systems under associating conditions the open association model (OAM) of Elias had already been demonstrated to give the best description of the scattering data.[5,6]

For a system having a polydispersity index, PI (PI = $\bar{M}_w/\bar{M}_n$), an apparent weight-average molar mass is defined as in

$$(\bar{M}_w)_{app} = \{[(\bar{M}_w)_0]^2 + 4000\mathrm{PI}(\bar{M}_w)_0 C_p\}^{1/2} \qquad (1)$$

where $(\bar{M}_w)_0$ is the weight-average molar mass of the nonassociating species (at infinite dilution) and K_{app} is the apparent association constant of the process. As in the original treatment of Elias,[2] K_{app} is supposed to be the same for each association step, independent of the actual overall extent of polymer association. We consider it as an apparent association constant, inasmuch as it will be treated as a phenomenological thermodynamic parameter, the absolute value of which and the dependence on temperature, ionic strength, and other physicochemical parameters of which will not be compared with the values calculated a priori on the basis of any particular model.

By substitution of $\bar{M}_w$ with $(\bar{M}_w)_{app}$ for an associating system, the familiar equation for the concentration dependence for the scattering at $\theta \to 0°$

$$\frac{KC_p}{R_\theta}\Big|_{\theta = 0°} = \frac{1}{\bar{M}_w} + 2A_2C_p \qquad (2)$$

takes the form:

$$\frac{KC_p}{R_\theta}\Big|_{\theta = 0°} = \frac{1}{(\bar{M}_w)_{app}} + 2A_2C_p = \frac{1}{[(\bar{M}_w)_0^2 + 4000K_{app}\mathrm{PI}(\bar{M}_w)_0 C_p]^{1/2}} + 2A_2C_p \qquad (3)$$

Equation 3 is formally a three-parameter equation, namely $(\bar{M}_w)_0$, K_{app}, and A_2. However, when the same polymer sample is used both in nonassociating and in different associating conditions, then the value of $(\bar{M}_w)_0$ has to be consistently the same for all cases. Under such circumstances, eq 3 will simplify to a two-parameter equation (i.e., K_{app} and A_2), $(\bar{M}_w)_0$ having been fixed to the value of the nonassociated form at $C_{p\to 0}$.

Results and Discussion

1. Characterization of the Association Process. In the series of $\bar{M}_w$ data reported by Slootmaekers et al.[3] as a function of the concentration of NaI in water at 25 °C, the one measured at the highest value of the concentration of added salt ([NaI] = 0.20 M) is 391 000. The ratio of the value of $\bar{M}_w$ under such conditions over the mean value of the unassociated polymer ($\langle\bar{M}_w\rangle_5$ = 328 500; see Table 1c of ref 1) is 1.19.

Such a difference of molar mass of about 20% in excess with respect to the mean value of the isolated polymer chain is beyond the accuracy limits of the technique, pointing to the existence of an ionic-strength-induced association process. This process is then expected to start for concentrations of iodide ions >0.15 M, for which full conformational ordering can be achieved without any change of the average molar mass.[1]

It was then decided to verify whether this finding could be confirmed in a series of experiments in the total integrated light-scattering (TILS) mode, using a LALLS setup, which is particularly sensitive to the presence of even minor amounts of associated material. All experimental conditions were the same as those described in ref 1, except for the values of the molar concentration of NaI, which were 0.20 and 0.30 M, respectively.

Table 1. Macromolecular Parameters of κ-Carrageenan in NaI (Ordered Form) at 22 °C; Solutions Were Prepared According to Ref 3

a. Results of a Linear Fit of the Reduced Inverse Scattering Data as a Function of C_p Obtained by Use of LALLS

ionic strength (M)	correlation coefficient	$\bar{M}_w \times 10^{-3}$	$A_2 \times 10^3$ (mol·mL·g^{-2})	$\chi^2 \times 10^{14}$
0.20	0.981	207.1 ± 1.2	0.88 ± 0.05	0.13
0.20	0.753	205.6 ± 3.7	1.42 ± 0.26	1.35
0.30	0.730	174.6 ± 2.7	6.10 ± 0.26	1.04

b. Results of the Fit with the OAM of Elias of the Reduced Inverse Scattering Data as a Function of C_p Obtained by Use of LALLS

ionic strength (M)	$\bar{M}_w \times 10^{-3}$	$A_2 \times 10^3$ (mol·mL·g^{-2})	$K_{app} \times 10^{-5}$	$\chi^2 \times 10^{14}$
0.20	204.6 ± 2.2[a]	1.79 ± 0.33	0.25 ± 0.01	0.11
0.20	187.1 ± 1.1[a]	4.38 ± 0.69	1.25 ± 0.56	1.10
0.30	160.6 ± 5.9[a]	3.83 ± 0.51	0.96 ± 0.29	0.44
0.20	156.5[b]	3.73 ± 0.34	2.17 ± 0.35	5.75
0.20	156.5[b]	5.51 ± 0.27	2.79 ± 0.22	1.50
0.30	156.5[b]	4.12 ± 0.22	1.15 ± 0.82	0.38

[a] Three-parameter fit. [b] Two-parameter fit.

In the polymer concentration range from about 5.0 × 10^{-5} g mL^{-1} to about 5.0 × 10^{-4} g mL^{-1}, linear plots of (KC_p/R_θ) were recorded, the linear fitting parameters of which are reported in Table 1a. Both $\bar{M}_w$ data fall by >10% above the mean value of $\bar{M}_w$ of the unassociated polymer (value indicated as $\langle\bar{M}_w\rangle_7$ in Table 1e of ref 1). Therefore the presence of an association equilibrium of κ-carrageenan for aqueous [NaI] = 0.20 M is clearly confirmed. The scattering data were analyzed in the framework of the OAM by Elias[2] as described in the Experimental Section. Application of eq 3 to the data and given the known value of the polydispersity index, PI = 2.15, the value of the weight-average molar mass of the fundamental associating unimer, $(\bar{M}_w)_0$, the value of K_{app}, and the limiting value of A_2 were determined. They are reported in Table 1b. The three-parameter fit is excellent, as indicated by the low values of the sum of the squares of the residuals, χ^2, and even more so by the fact that the values of $(\bar{M}_w)_0$ were found to be very close to the mean value of $\bar{M}_w$ of the single-stranded polymer in its disordered form and in the fundamental ordered conformation. It was then an obvious further choice not to use $(\bar{M}_w)_0$ as a fitting parameter, but to set it equal to the value of $\langle\bar{M}_w\rangle_7$. The fit of the data points is still excellent, as confirmed both by the low values of χ^2 taking into account that it is a two-parameter fit, and by inspection of the plots of Figure 1a and b, respectively. The curves of this figure have been drawn using the respective new sets of the two parameters, K_{app} and A_2, which are reported in Table 1b. The successful application of the OAM (still aware of its limitations) is a strong diagnostic evidence for the ion-induced association of κ-carrageenan even in the presence of an allegedly "solubilizing" anion like iodide, for [NaI] ≥ 0.20 M. Iodide anions do not seem to violate the general observation that, in the case of carrageenans, the simultaneous presence of isolated chains in the fundamental ordered conformation ("*intramolecular* ordering") and of associated forms of stretches thereof (in the simplest case, dimers; "*intermolecular* ordering") is a very common situation. A "window" in the conformational phase diagram, in which only the *intramolecular* fundamental ordered form can be isolated,

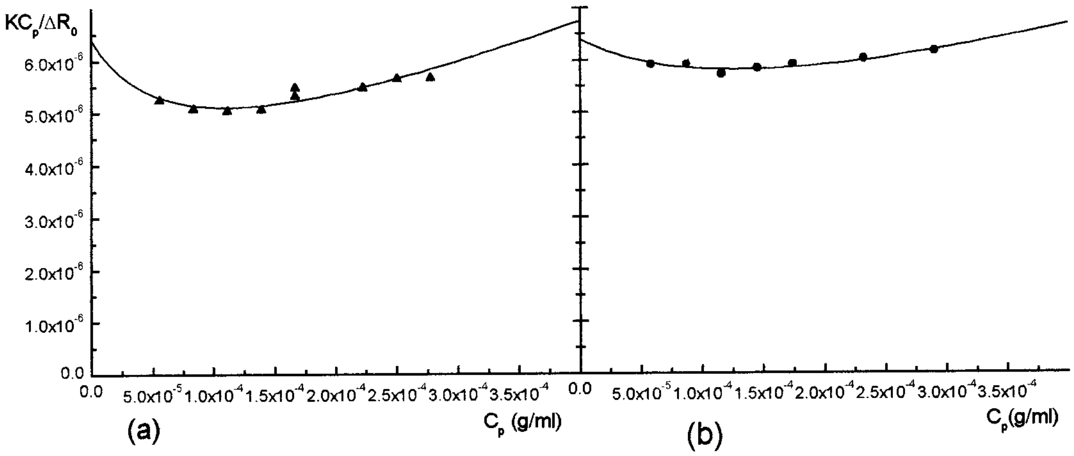

Figure 1. Dependence of the inverse of the reduced scattering intensity at zero angle on the polymer concentration of κ-carrageenan in NaI fitted with the OAM. a: [NaI] = 0.20 M; b: [NaI] = 0.30 M.

Table 2. Weight-Average Molar Mass and Apparent Association Constant of κ-Carrageenan in 0.20 M LiI; Data Pertaining to Cooling and Heating Cycles from Ref 7

temp (°C)	mode	$\bar{M}_w \times 10^{-3}$	$\rho(T) = \bar{M}_w/(\bar{M}_w)_0$	$K_{app} \times 10^{-5}$
25	heat	393 ± 20	1.99	1.22
	cool	476 ± 20	2.42	1.98
35	heat	375 ± 15	1.90	1.07
	cool	420 ± 15	2.13	1.45
42	heat	355 ± 7	1.80	0.92
	cool	357 ± 10	1.81	0.93
50	heat	290 ± 8	1.47	0.48
	cool	303 ± 8	1.54	0.56
60	heat	207 ± 9	1.05	0.043
	cool	210 ± 9	1.07	0.056
70	heat	197 ± 5	1	

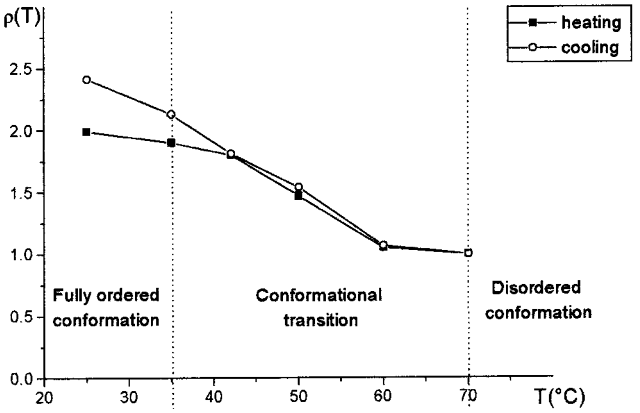

Figure 2. Temperature dependence of association parameters of κ-carrageenan in 0.20 M LiCl. Change of the weight-average molar mass ratio, *ρ*(*T*), as a function of temperature, *T*, in heating and cooling cycles: data taken from Figure 5 from ref 7.

may be a condition difficult to achieve. Iodide ions allow reaching such a goal under conditions less limited than those often pertaining to other simple electrolytes, as has been amply illustrated in the previous paper.[1] However, the present findings demonstrate that it would be quite wrong to believe that the iodide ion per se would permit the loss of chain association under *all* circumstances.

2. Temperature-Induced Association. Recently, an extensive report on the determination of $\bar{M}_w$ of κ-carrageenan in iodide aqueous solution based on the use of size-exclusion chromatography (SEC)-LALLS has been reported.[7] The work has the merit to be, together with that of Ueda et al.,[8] one of the two first systematic studies the molar mass of κ-carrageenan as a function of temperature, following the related but not coincident processes of conformational transition and of interchain association. The $\bar{M}_w$ of their sample has been measured at different temperatures, in a thermal cycle from 25 °C to 70 °C, both in the cooling and in the heating mode. The main point is that the concentration of added iodide used has been 0.20 M throughout. On the basis of the results described in the preceding paragraph, a substantial increase of $\bar{M}_w$ was expected for [LiI] = 0.20 M at temperatures around 25 °C, because of chain association. This is precisely what can be seen from the molar mass data, here reported in Table 2 and plotted in Figure 2 as $\rho(T)$: $\rho(T)$ is the ratio between the weight-average molar mass at the temperature T, $\bar{M}_w(T)$, over that of the single-stranded disordered form, $(\bar{M}_w)_0$. Parallel to the onset of conformational ordering as monitored by optical activity (see Figure 5 of ref 7), $\bar{M}_w$ starts to increase in a monotonic way. However, it keeps increasing even below the value of temperature, T = 35 °C, at which the chiro-optical data indicate the complete attainment of conformational ordering. This is a clear indication that the association process is a continuous "open" process. No theoretical limit seems to be suggested by the trend of the experimental $\bar{M}_w$ values, such as, for example, it would be expected for an exact doubling upon formation of an intertwined double helix. In this respect, no "general" conclusion should be drawn as to the molecularity of the fundamental ordered conformation because the reported experimental results pertain only to a very specific set of the physicochemical variables, exclusively limited to associative conditions.

Although the experiments of Hjerde et al.[7] were not performed for different values of polymer concentration, use can be made of eq 4:

$$K_{app,T} = \frac{(\bar{M}_{w,T})^2 - [(\bar{M}_w)_0]^2}{4000(\bar{M}_w)_0 PIC_p} \tag{4}$$

to obtain the value of K_{app}, according to Elias' model. The necessary data are easily available: $(\bar{M}_w)_0$ and PI have been taken as equal to those reported for the highest temperature pertaining to the disordered, non-associated polymer; the values of $\bar{M}_{w,T}$ were taken from Table 2 of ref 7 for each value of T; the polymer

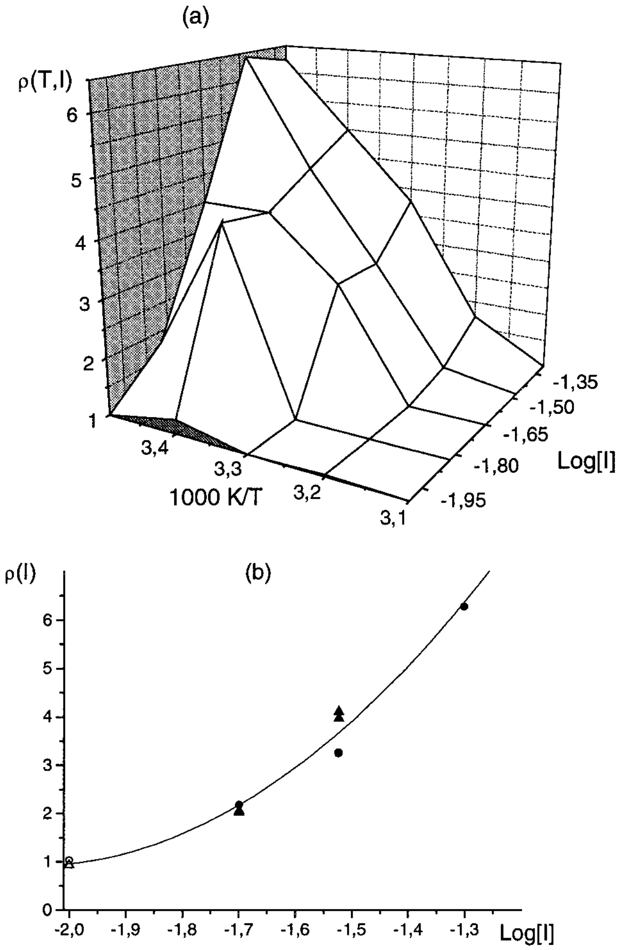

Figure 3. (a) Three-dimensional plot of the weight-average molar mass ratio, $\rho(T,I)$, as a function of both the inverse of the absolute temperature, T, and the logarithm of the ionic strength, I; data taken from ref 8, after interpolation. (b) Dependence of the weight-average molar mass ratio, $\rho(I)$, on the logarithm of the ionic strength, I. All data pertain to full conformational ordering, at the temperature of 10 °C. Open symbols: data from Table 1f of ref 1; full symbols: data from Tables 1 and 2 of ref 8; triangles: sample S-1; circles: sample S-2. The line has been drawn to help the eye.

concentration was taken equal to 8.6×10^{-4} g mL^{-1} throughout.[7] The results, reported in Table 2, are very close to those reported in Table 1b. The tendency to association clearly grows parallel to conformational ordering in a continuous way.

A similar *continuous* trend of association has been reported by Ueda et al.[8] for aqueous solutions of two κ-carrageenan samples in the presence of KCl. Those data, obtained by use of a SEC-LALLS setup, are very interesting because they pertain to a variation range of *both T and I*. The values of the ratio $\rho(I,T)$ are reported in a three-dimensional graph in Figure 3a as a function of the logarithm of the molarity of the supporting electrolyte (identified with the ionic strength) and of the inverse of the absolute temperature, which are the usual independent variables for the conformational phase diagrams of biopolyelectrolytes. It can also be seen that the increase of $\bar{M}_w$ of the ordered associated forms with [KCl] is continuous upon decreasing T *and* increasing I with no evidence of leveling off at a special, integral value such as two (for a double helix) or three (for a triple helix). K_{app} values evaluated for the various (I,T) pairs are of the same order of magnitude as those above reported in Table 2: the results pertaining for the case of $T = 10$ °C are reported in Table 3 for both samples; the corresponding plot of $\rho(I)$ vs $\log(I)$ is reported in Figure 3b. As far as the continuous association process induced by K$^+$ ions is concerned, the trend of the molar mass data of Ueda et al.[8] is in perfect agreement with the polyelectrolyte analysis based upon microcalorimetric results.[9]

Table 3. Weight-Average Molar Mass and Apparent Association Constant of κ-Carrageenan at 10 °C in Aqueous KCl; Data from Ref 8

ionic strength (M)	sample	$\bar{M}_w \times 10^{-3}$	$\rho(I) = \bar{M}_w/(\bar{M}_w)_0$	$K_{app} \times 10^{-5}$
0.020	S-1	117	2.02	0.440
0.020	S-1	119	2.05	0.456
0.030	S-1	238	4.10	2.157
0.030	S-1	230	3.97	2.007
0.020	S-2	76	2.18	0.231
0.030	S-2	113	3.25	0.585
0.050	S-2	218	6.26	2.343

3. Effect of the Preparation Procedure on the Values of $\bar{M}_w$. It has been amply demonstrated by use of both TILS[3] and SEC data[7] obtained with LALLS equipment that 0.20 M represents a concentration value of the iodide salt form of an alkali metal ion in which association of ordered chains of κ-carrageenan occurs, at around room temperature. Moreover, the association process has been confirmed by assessing its thermal evolution at constant polymer concentration from data obtained with a SEC-LALLS setup,[7] indicating that the chain association starts already around 60 °C in 0.20 M LiI. Besides the data of Slootmaekers et al.,[3] who made use of wide-angle laser light scattering (WALLS) in the TILS mode, the only other set of data obtained in aqueous [NaI] = 0.20 M that took into account also the angular dependence of scattering are those of Viebke et al.[4]

Following the procedure described in the Experimental Section, we got the proper values of $(KC_p)/R_\theta|_{\theta = 0°}$. Linear regression analysis of the inverse reduced intensity data vs C_p gave a fit with $\bar{M}_w = 760\,000$, $A_2 = 1.313 \times 10^{-3}$ mol mL g^{-2}, but χ^2 was as high as 5.784×10^{-15}. Inspection of the plot revealed an upward curvature, which is diagnostic for association processes. Therefore we decided to analyze the data by use of the OAM. The value of the molar mass of the unimeric form, $(\bar{M}_w)_0$, was fixed to 310 000, that is, the mean between the $\bar{M}_w$ of the same sample of κ-carrageenan in the disordered conformation obtained in the parallel experiment in NaNO$_3$ (324 000) and the value for the same sample as determined by SEC multiangle laser light scattering (MALLS) experiment (296 000). The value of PI was taken as 2.7 from a similar sample used in the experiments reported in ref 10. The marked reduction of χ^2 ($\chi^2 = 1.80 \times 10^{-15}$) was a clear indication of the validity of the choice of the model: the graphical result of the fitting procedure is reported in Figure 4. The value of A_2 was found to be 1.48×10^{-3} mol mL g^{-2}, whereas K_{app} as 3.72×10^5 (see Table 4).

A summary of all the different $\bar{M}_w$ and K_{app} data obtained in 0.20 M alkali metal iodides is given in Table 4. The values of K_{app} for the Na$^+$ form of κ-carrageenan are larger than those of the Li$^+$ salt form: this is not very surprising since lithium ions are known to produce a lower association tendency of κ-carrageenan than sodium ions. If compared with the other results that point to the presence of a polymer association, the results of ref 4 seem to produce a higher level of

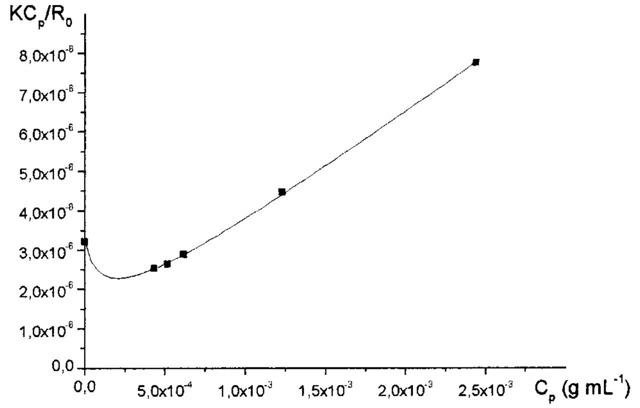

Figure 4. Dependence on polymer concentration of the reduced scattering intensity at $\theta = 0°$ of κ-carrageenan in [NaI] = 0.20 M. Data from ref 4, fitted with the OAM.

Table 4. Weight-Average Molar Mass and Apparent Association Constant of κ-Carrageenan at 22 °C in Aqueous Solution in the Presence of 0.20 M Alkali-Metal Iodides

reference	alkali-metal cation	$\bar{M}_w \times 10^{-3}$	$A_2 \times 10^3$ (mol·mL·g^{-2})	$K_{app} \times 10^{-5}$
this work	Na$^+$	156.5	3.73	2.17
this work	Na$^+$	156.5	5.51	2.79
7	Li$^+$ (cooling)	197	not given	1.98
7	Li$^+$ (heating)	197	not given	1.22
4	Na$^+$	310	1.48	3.72

Table 5. Macromolecular Parameters of κ-Carrageenan in 0.15 M NaI at 22 °C as Determined by LALLS; Solutions Prepared Following the Protocol of Ref 4

fitting function	$\bar{M}_w \times 10^{-3}$	$A_2 \times 10^3$ (mol·mL·g^{-2})	$K_{app} \times 10^{-5}$	$\chi^2 \times 10^{14}$
linear	202.0 ± 5.1	2.70 ± 0.29		0.73
OAM[a]	126.6 ± 13.6	5.97 ± 0.37	2.85	0.94
OAM[b]	156.5[c]	5.12 ± 0.38	1.45	1.75

[a] Three-parameter fit. [b] Two-parameter fit. [c] Fixed value of $(\bar{M}_w)_0$.

association. From the comparison of the respective procedures of solution preparation, it seems very reasonable to attribute such a discrepancy to that experimental step. At variance with the case of dissolving κ-carrageenan into "disordering" 0.15 M NaCl, which was demonstrated to allow the chains to reach a complete molecular dispersion,[1] the chain-ordering effect of 0.20 M NaI is likely to "kinetically trap" some part of the polymer into an aggregated form. The latter one is different from that expected for the true thermodynamic equilibrium, and a higher value of K_{app} will then be produced.

It was then decided to repeat their procedure of solution preparation[4] to assess the aggregational effect of the ordering conditions against the complete polymer dissolution. A concentration of NaI of 0.15 M was chosen, that is, intermediate between the data obtained in the TILS mode ([NaI] = 0.20 M) and in the SEC-MALLS mode ([NaI] = 0.10 M).[4] Moreover, it corresponded to the molarity of NaCl for which the parallel experiment was made in disordering conditions (see Table 2 of ref 1). The $\bar{M}_w$ was calculated by TILS-LALLS; the relevant parameters are reported in Table 5 and the experimental results are reported in Figure 6. An apparent molar mass of 202 000 was obtained from the linear extrapolation of the data points with $C_p > 1.61 \times 10^{-4}$ g·mL^{-1} (see curve a of Figure 6). This is 29% higher than the $\bar{M}_w$ in the disordered form and in the ordered form obtained following the conventional procedure avoiding the "ionic-shock" (i.e., 156 500). However, the exceptional sensitivity of the LALLS method allowed us to investigate into a still more dilute range of concentrations. On decreasing C_p down to 6.20×10^{-5} g mL^{-1}, an upward curvature started to be apparent as can be seen from the results shown in curve b of Figure 6. The application of eq 3 to the data produced the values of the parameters reported in Table 5. The obvious conclusion of such comparative experiments is that the procedure of preparation of aqueous solutions of carrageenans is a very delicate operation. The "ionic-shock" procedure, that is, throwing the freeze-dried material into a (high concentration) of order-inducing electrolytes such as the monovalent counterion iodides, was shown to be a method producing a substantial level of chain *aggregation*.

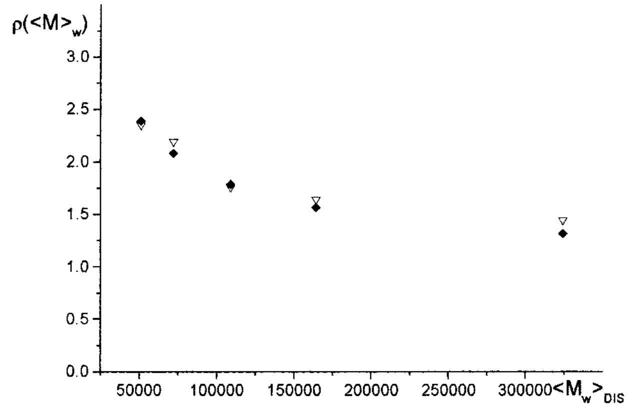

Figure 5. Dependence of the weight-average molar mass ratio, $\rho(\bar{M}_w)$, between the weight-average molar mass in the ordered conformation ("ord") over that in the disordered ("dis") conformation, on the average molar mass in the disordered conformation, $(\bar{M}_w)_{dis}$, for different values of $(\bar{M}_w)_{dis}$. ▽: data taken from ref 4, Table 2; ◆: calculated data using eq 7 with $K' = 12\,000$.

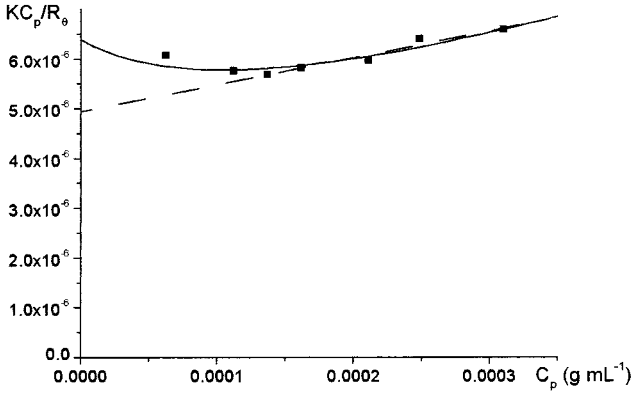

Figure 6. Dependence of the inverse of the reduced scattering intensity on the polymer concentration of κ-carrageenan in [NaI] = 0.15 M, following the preparation method of ref 4. Curve a: Linear fit of data for the polymer concentrations between 1.61×10^{-4} g·mL^{-1} and 3.10×10^{-4} g·mL^{-1}, dashed line; curve b: fit according to the OAM, including all the polymer concentrations, full line.

Similar considerations can arise from the analysis of the $\bar{M}_w$ data reported in ref 4 on a series of κ-carrageenan batches that were obtained by ultrasonication of the original sample for increasing times to obtain samples with decreasing molar mass. SEC-MALLS analysis was performed on such samples, both in the disordered conformation (0.10 M NaNO$_3$) and in the ordered one (0.10 M NaI). The latter ionic composition was shown to induce the ordered conformation of κ-carrageenan with no change of molar mass with respect to the single-stranded disordered form.[1] A monotonic decreasing curve is obtained for the ratio of the $\bar{M}_w$ in the ordered conformation, $(\bar{M}_w)_{ord}$, over the $\bar{M}_w$ in the disordered conformation, $(\bar{M}_w)_{dis}$, as a function of $(\bar{M}_w)_{dis}$. The value of this ratio is not far from three at the lowest limit and at the highest limit almost reaches the value of one. The results are reported as downward open triangles (▽) in Figure 5.

On the basis of the OAM, it has been stated[2] that, for a given set of values of PI and, in particular, of the *specific* polymer concentration (C_p in g mL^{-1}), the apparent association constant determined for samples of *decreasing* $(\bar{M}_w)_0$ is actually expected to be *increasing*. This effect trivially derives from the fact that the lower $(\bar{M}_w)_0$, the higher is the *molar* polymer concentration for a given, fixed value of the *specific* concentration, which is expressed on a *mass* scale. Therefore, the effect of mass-action law will be to shift the association equilibrium to higher values.

It was decided to apply such a treatment to the data of ref 4, using the experimental values of $(\bar{M}_w)_{dis}$ from Table 2 of ref 7 and for C_p the value of 0.005 g mL^{-1}. Equation 5, straightforwardly derived from eq 3,

$$(\bar{M}_w)_{ord} = [(\bar{M}_w)^2_{dis} + 4000K_{app}PI(\bar{M}_w)_{dis}C_p]^{1/2} \qquad (5)$$

allows for an easy calculation of the values of $(\bar{M}_w)_{ord}$ for any value of C_p. Although it is independently parametric both for K_{app} and for PI, one can use the unresolved parameter K' ($K' = K_{app}PI$) for the calculation of $(\bar{M}_w)_{ord}$ as a function of C_p. In fact, in the absence of PI data for all the different $(\bar{M}_w)_{dis}$ cases, our aim was just to assess the validity of the open association hypothesis. Using for K' the value of 12 000, the calculated points marked as diamonds in Figure 5 were obtained, showing an excellent agreement with the experimental values. In spite of the merely illustrative nature of the calculations, it can be concluded that an open chain-association process is taking place driven by an increasing molar concentration of polymer, as predicted by the theory. Therefore no conclusion whatsoever can be drawn on the molecularity (and even less on the topology) of the *fundamental associated ordered* form(s) from those experiments.

A recent paper by Wittgren et al.[10] provided additional $\bar{M}_w$ data that can be used to further illustrate the importance of the preparation method. Using a combination of flow field-flow fractionation and MALLS, a sample of κ-carrageenan was studied in different 0.10 M salt solutions, prepared according to the procedure of ref 4.

By application of eq 5 to their data together with the parameters given in ref 10 [i.e., $(\bar{M}_w)_{ord} = 690\,000$; $(\bar{M}_w)_{dis} = 370\,000$; $(PI)_{dis} = 2.7$: $C_p = 0.00523$ g mL^{-1}], a value of 16 200 is calculated for K_{app}, and hence a value of 43 800 for the unresolved parameter K'. A comparison with the previously obtained value for the sample of ref 4 having a similar molar mass [$(\bar{M}_w)_{dis} = 324\,000$] in the same NaI concentration (0.10 M) is useful: in that case K' is found to be 12 000. Given the very high similarity between the two samples, it is also likely that the two PI values are very similar, making the direct comparison quite realistic. As a consequence, the bare ratio of the two values of K' comes to indicate that the tendency to association of the sample of ref 10 is *about three times larger* than that of ref 4. The Materials and Methods section of ref 10 reveals that in this case the heating at 80 °C under stirring has been carried out *for 15 min only*, as compared with 30 min reported in ref 4. The difference in K_{app} is then clearly attributed to insufficient time and energy allowed for reaching a truly molecularly disperse solution.

Conclusions

From the whole of the above analysis of molar mass data it appears evident that κ-carrageenan is very sensitive to association phenomena, which may even reach the stage of irreversible aggregation.

There is no risk of overestimating the importance of a careful design of the physicochemical experiment on carrageenans insofar as their reversible tendency to associate may be concerned, mainly as to the value of the ionic strength. Many interesting experiments, aimed at shedding light onto the long-lasting problem of the molecularity of the fundamental ordered form of carrageenans, inadvertently were carried out in conditions of extensive chain association due to the high value of the molarity of the supporting simple electrolyte. They include, for example, SEC-LALLS determinations of molar masses,[7] stopped-flow kinetics of salt-jump-induced conformational ordering,[11] and depolymerization kinetics studied with a variety of techniques.[12-14] In all the quoted cases the molar concentration of iodide was 0.20 M (for ref 11, at least in the initial mixing process); in other cases additional HCl present as reagent substantially contributed to increase the ionic strength even more.[12-14]

Another major source of discrepancy turned out to be related to the preparation method that was sometimes used for κ-carrageenan solutions in the ordered conformation.[4,10] The process of freeze-drying is well known to produce an extensive chain aggregation, both for nonionic and for ionic polysaccharides. Exposing the freeze-dried material to an ionic shock, especially if the latter is produced by the high value of the concentration of an order-inducing ion (like iodide), does not allow the macromolecules to reach a state of thermodynamic equilibrium in a molecularly disperse form.

Acknowledgment. The present research project has been financially supported by FWO and by the University of Trieste, and by POLY-biòs Research Center from the scientific standpoint. K.B. is grateful to the K.U. Leuven for the doctoral fellowship and to the Department of Biochemistry, Biophysics and Macromolecular Chemistry of the University of Trieste and POLYtech S.C.r.l. for hospitality in their laboratories.

References and Notes

(1) Bongaerts, K.; Reynaers, H.; Zanetti, F.; Paoletti, S. *Macromolecules*, in press.

(2) Elias, H. G. Association and Aggregation as Studied via Light Scattering. In *Light Scattering from Polymer Solutions*; Huglin, M. B., Ed.; Academic Press: London, 1972; Chapter 9, pp 397−457.

(3) Slootmaekers, D.; De Jonghe, C.; Reynaers, H.; Varkevisser, F. A.; Bloys van Treslong, C. J. *Int. J. Biol. Macromol.* **1988**, *10*, 160−168.

(4) Viebke, C.; Borgström, J.; Piculell, L. *Carbohydr. Polym.* **1995**, *27*, 145−154.

(5) Vanneste, K. Ph.D. Thesis, Katholieke Universiteit Leuven, Heverlee, Belgium, 1993.

(6) Denef, B. Ph.D. Thesis, Katholieke Universiteit Leuven, Heverlee, Belgium, 1997.

(7) Hjerde, T.; Smidsrød, O.; Christensen, B. E. *Biopolymers*, in press.

(8) Ueda, K.; Itoh, M.; Matsuzaki, Y.; Ochiai, H.; Imamura, A. *Macromolecules* **1998**, *31*, 675−680.

(9) Paoletti, S.; Delben, F.; Cesàro, A.; Grasdalen, H. *Macromolecules* **1985**, *18*, 1834−1841.

(10) Wittgren, B.; Borgström, J.; Piculell, L.; Wahlund, K.-G. *Biopolymers* **1998**, *45*, 85−96.

(11) Austen, K. R. J.; Goodall, D. M.; Norton, I. T. *Biopolymers* **1988**, *27*, 139−155.

(12) Hjerde, T.; Smidsrød, O.; Christensen, B. E. *Macromolecules* **1998**, *31*, 1842−1851.

(13) Hjerde, T.; Smidsrød, O.; Christensen, B. E. *Carbohydr. Res.* **1996**, *288*, 175−187.

(14) Hjerde, T.; Kristiansen, T. S.; Stokke, B. T.; Smidsrød, O.; Christensen, B. E. *Carbohydr. Polym.* **1994**, *24*, 265−275.

MA981204R