On the Molar Mass of κ-Carrageenan in the Course of Conformational Transition from the Disordered to the Fundamental Ordered Form

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: Weight-average ($\bar{M}_w$) molar mass data were obtained by light scattering on aqueous solutions of a sample of κ-carrageenan with a relative molar mass of about 157 000. They were compared with literature data of several samples, having $\bar{M}_w$ values from 35 000 to 720 000, using a variety of methods. Temperature and concentration of supporting 1:1 electrolyte were chosen so as to span across the disorder-to-order conformational transition, monitored by the change of optical activity. The present comparative study indicates that the single helix is the fundamental ordered (secondary) conformation of κ-carrageenan in aqueous solution. This conclusion appears to be universally valid on the basis of a very large number of cases, including polymer samples of different average molar mass, in the presence of different counterions and co-ions, and at different temperatures. The single-helical structure is the conformational "building-block" of a higher ("tertiary") ordered structure that stems from the association of (at least) two of such secondary structures according to a topology that cannot be determined by light-scattering data only.

Introduction

Carrageenans are red-algae galactans, sulfated to a different extent.[1] κ-Carrageenan is one of the most widely known and commercially exploited members of that polysaccharide family. Its ideal copolymeric repeating unit is reported in Figure 1. One of the most peculiar features of κ-carrageenan is related to its ability to give rise to ionotropic gels, in particular with alkaline metal counterions of high atomic number. Such gels are thermoreversible, like those of the companion molecule ι-carrageenan and of the chemically and phylogenetically related polysaccharide agarose. The detailed molecular interpretation of the gelation mechanism of κ-carrageenan has been highly controversial over the past 20 years.

Whereas the hypothesis that a disorder-to-order conformational transition underlying the physical gelation mechanism[2] through the formation of conformationally ordered junctions has been almost universally accepted, there is still an active debate as to the nature of the fundamental ordered conformation of the biopolymer. According to some students of κ-carrageenan[2−5] such a conformation is a coaxial double helix. The alternative line of structural interpretation runs from very early work[6] to some of the most recent contributions[7] suggesting that the fundamental ordered conformation is a single helix, with the same conformational features as one strand of the proposed double helix.

The latter point implies that data obtained by techniques that are strongly sensitive only to the local conformation, such as, for example, optical activity, would be of no help in a discrimination among the two models. The pronounced tendency of the polymer toward intermolecular association (and often to irreversible aggregation) had enormously impaired a conclusive assessment of this issue by use of, for example, light-scattering data. This paper is providing new data on the molar mass of κ-carrageenan under conditions that favor the fundamental ordered conformation *without* association. In addition, it contributes to a comparative analysis of a wealth of literature data, which are shown to support consistently the present findings. A following paper[8] is dedicated to the study of the intermolecular association of κ-carrageenan in aqueous solutions under different physicochemical conditions.

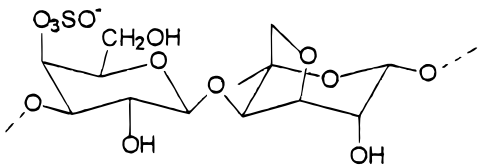

Figure 1. Scheme of a κ-carrageenan disaccharide repeating unit.

Experimental Section

Materials. The κ-carrageenan sample used was from Sigma Chemical Co., Type III (code 37 050). It was dissolved in highly purified water by stirring under heating, and fractionated by dropwise addition to 2-propanol (C. Erba P. A.) under vigorous stirring. The precipitate was rinsed thoroughly with ultrapure water (Milli-Q-Reagent Grade Water Systems, Millipore) and

* Corresponding author.

10.1021/ma981203z CCC: $18.00
Published on Web 01/22/1999

dissolved. It was first dialyzed against 0.20 M NaCl and then extensively against water before freeze-drying. The compositional purity of the sample was analyzed by ^{1}H NMR spectroscopy; not more than 5% ι-carrageenan segments were found to be present as impurity. NaCl and NaI were analytical grade products from Carlo Erba S.p.A. and ultrapure water was always used.

Methods. Low-angle laser light scattering (LALLS) measurements were performed with a KMX-6 (Thermo Separation Products) equipped with a Ne−He laser (λ = 632.8 nm). Readings were made at 22 °C in the standard measuring cell with a sample volume of 150 μL and a scattering volume of 0.1 μL. Solutions were filtered into the cell through 45-μm Teflon (PTFE) filters using a syringe pump. The intensity of the scattered light was registered using the 6÷7-degree annulus and the 0.2-mm fieldstop. The photomultiplier signal was registered on a strip-chart recorder while allowing the solutions to flow through the cell at a low flow rate. After correction for instrumental, optical, and geometrical constants, the Rayleigh ratio, R_θ, was determined directly from the ratio between the signal from the photomultiplier caused by light scattered from the sample at an angle θ and the signal caused by the incident beam transmitted through the sample. The weight-average molecular weight, $\bar{M}_w$, and the second virial coefficient, A_2, were evaluated using the familiar eq 1 for the linear regression of the data under nonassociating conditions.

$$\frac{KC_p}{R_\theta} = \frac{1}{\bar{M}_w} + 2A_2C_p \qquad (1)$$

K is the optical constant in the light-scattering equation and C_p is the polymer concentration in g mL^{-1}. No extrapolation to zero angle was carried out, as usual in LALLS, as the median scattering angle used was close to zero. For the refractive index increment, dn/dc, needed to determine K, the value of 0.150 mL g^{-1} was taken both for NaCl and for NaI.[9] The molecular weight distribution (MWD) curve of the polymer sample was obtained by a separate size-exclusion chromatography (SEC)-LALLS experiment. The chromatographic system was the same as previously described.[10] κ-Carrageenan was dissolved in 0.15 M NaCl , which was also the chromatographic eluent. The MWD curve gave the following parameters: $\bar{M}_w$ = 153 000, $\bar{M}_n$ = 71 000; the polydispersity index, PI, was then equal to 2.15. Polymer solutions were prepared according to the following procedure: freeze-dried purified κ-carrageenan was suspended in ultrapure water under vigorous stirring at room temperature for at least 12 h. Concentrated salt solutions were added dropwise to the polymer salt-free aqueous solution under stirring, to avoid nonequilibrium aggregation until the desired salt molarity and C_p was reached.

Results and Discussion

1. Results Obtained Using Extrapolation Procedures to Zero Angle. The earliest reported attempts at determining the $\bar{M}_w$ of κ-carrageenan in the ordered conformation date back to the 1970s.[11,12] The experiments were carried out at around room temperature in the presence of the order-inducing (but also gel-forming) K^+ cation. The reported values of $\bar{M}_w$ were very large as compared with those in the disordered conformation, indicating that under such conditions an extensive association/aggregation process was taking place.

In 1980 Smidsrød et al.[13,14] showed on the more highly sulfated polysaccharide ι-carrageenan that in aqueous solutions containing LiI it was possible to observe the complete disorder-to-order conformational transition without a concomitant increase of molar mass (measured as $\bar{M}_n$). Moreover, the specific optical activity, [α], of the ordered conformation was not concentration dependent, as should be expected for the formation of an intertwined double-stranded helix[15] from isolated disordered chains. This paper marked the start of a long-lasting debate that encompassed both κ- and ι-carrageenan, and to some extent agarose as well. Soon after, Grasdalen and Smidsrød published very similar results for κ-carrageenan,[16] showing that at 25 °C the experimental $\bar{M}_n$ values for two samples, differing by a factor of about five as to their average molar mass, did not change on passing from the disordered conformation [in aqueous tetramethylammonium (TMA) chloride 0.15 M] to the completely ordered form (when the solvent was aqueous 0.15 M TMAI). Binding of iodide anions to the polyanionic chain of κ-carrageenan was also demonstrated on the basis of NMR evidence. In a following paper,[17] the corresponding constancy of $\bar{M}_w$, as determined by the use of wide-angle (WA) total integrated light-scattering (TILS) mode, for the sample of higher molar mass of their previous paper, was additionally demonstrated.

Those results are summarized in Table 1a, which lists also the ratio $\bar{M}_i/\langle\bar{M}_i\rangle$, of the proper molar mass average (i.e., either number or weight) for any given ordered conformation ($\bar{M}_i$) and the mean of all the corresponding average molar masses for the same polymer sample in all conformations, $\langle\bar{M}_i\rangle$.Table 1a−g show that taking only one overall mean for all values of $\bar{M}_i$ for a given polymer sample was a "statistical must", since in all cases the separate values of the mean of $\bar{M}_i$ for the polymer in the disordered form were extremely close to the corresponding mean value in the ordered conformation. In particular, their difference was *always significantly smaller* than the standard deviation of the two separate means and lower than the estimated relative accuracy in the determination of $\bar{M}_i$ (i.e., about 10%).

It should be stressed that, after careful testing of each set of data, this observation on molar mass constancy turned out to be valid for all other molar mass values hereafter described, and altogether reported in Table 1, from 1a to 1g.

In a parallel study[18] on a different sample of κ-carrageenan using mainly, but not exclusively, the WA-TILS technique, Smidsrød and Grasdalen demonstrated that the intramolecular nature of the disorder-to-order conformational transition of κ-carrageenan was not limited to iodide-containing aqueous solutions. It was thereby shown that by a proper choice of conditions, low temperature and high concentration of added 1:1 electrolyte, it was possible also in aqueous TMACl to obtain the same value for the $\bar{M}_w$ of the ordered conformation of κ-carrageenan as obtained for the disordered one (see Table 1b). The experimental WA-TILS data in some cases showed a marked curvature, but careful investigation at sufficiently low values of C_p allowed a safe and reliable extrapolation to both $\theta \rightarrow 0$ and $C_p \rightarrow 0$. However, the nonlinear Zimm plots obtained under conditions of high ordering (i.e., very high salt concentration) indicated that the intramolecular transition was dominant

Table 1. Number-($\bar{M}_n$) and Weight-Average Molar Mass ($\bar{M}_w$) Values of κ-Carrageenan under Different Conformational Conditions

a. Data from refs 16 ($\bar{M}_n$) and 17 ($\bar{M}_w$)

T (°C)	counterion	co-ion	ionic strength (M)	$\bar{M}_n \times 10^{-3}$	% order	$\bar{M}_n/\langle\bar{M}_n\rangle_1$
25	TMA^+	Cl^-	0.15	32	0	0.96
25	TMA^+	I^-	0.15	35	100	1.05
					$\langle\bar{M}_n\rangle_1 = (34 \pm 2) \times 10^3$	

T (°C)	counterion	co-ion	ionic strength (M)	$\bar{M}_n \times 10^{-3}$	% order	$\bar{M}_n/\langle\bar{M}_n\rangle_2$
25	TMA^+	Cl^-	0.15	150	0	1.00
25	TMA^+	I^-	0.15	150	100	1.00
					$\langle\bar{M}_n\rangle_2 = (150 \pm 0) \times 10^3$	

T (°C)	counterion	co-ion	ionic strength (M)	$\bar{M}_w \times 10^{-3}$	% order	$\bar{M}_w/\langle\bar{M}_w\rangle_3$
25	TMA^+	Cl^-	0.15	720	0	1.08
25	TMA^+	I^-	0.15	610	100	0.92
					$\langle\bar{M}_w\rangle_3 = (665 \pm 55) \times 10^3$	

b. Data from ref 18

T (°C)	counterion	co-ion	ionic strength (M)	$\bar{M}_w \times 10^{-3}$	% order	$\bar{M}_w/\langle\bar{M}_w\rangle_4$
20	TMA^+	Cl^-	0.05	500	0	1.01
20	Na^+	Cl^-	0.10	522	0	1.06
20	TMA^+	Cl^-	0.15	480	10	0.97
20	TMA^+	I^-	0.15	480	100	0.97
20	TMA^+	Cl^-	0.30	460	70	0.93
10	TMA^+	Cl^-	0.30	520	100	1.05
20	TMA^+	Cl^-	0.60	500	100	1.01
					$\langle\bar{M}_w\rangle_4 = (497 \pm 23) \times 10^3$	

c. Data from ref 9

T (°C)	counterion	co-ion	ionic strength (M)	$\bar{M}_w \times 10^{-3}$	% order	$\bar{M}_w/\langle\bar{M}_w\rangle_5$
25	Na^+	Cl^-	0.02	326	0	0.99
25	Na^+	I^-	0.02	360	0	1.10
25	Na^+	Cl^-	0.04	329	0	1.00
25	Na^+	I^-	0.04	337	4	1.03
25	Na^+	Cl^-	0.05	340	0	1.03
25	Na^+	I^-	0.05	300	11	0.91
25	Na^+	I^-	0.055	286	12	0.87
25	Na^+	Cl^-	0.07	352	0	1.07
25	Na^+	I^-	0.07	317	50	0.97
25	Na^+	Cl^-	0.10	322	0	0.98
25	Na^+	I^-	0.10	297	90	0.90
25	Na^+	I^-	0.10	362	90	1.10
25	Na^+	Cl^-	0.12	333	100	1.01
25	Na^+	I^-	0.12	361	100	1.10
25	Na^+	Cl^-	0.15	319	0	0.97
25	Na^+	I^-	0.15	351	100	1.07
					$\langle\bar{M}_w\rangle_5 = (329 \pm 13) \times 10^3$	

d. Data from ref 21

T (°C)	counterion	co-ion	ionic strength (M)	$\bar{M}_w \times 10^{-3}$	% order	$\bar{M}_w/\langle\bar{M}_w\rangle_6$
30	Na^+	Cl^-	0.20	452	0	0.99
60	Na^+	I^-	0.10	464	0	1.01
21	Na^+	I^-	0.10	462	100	0.99
					$\langle\bar{M}_w\rangle_6 = (453 \pm 6) \times 10^3$	

e. Data from this work

T (°C)	counterion	co-ion	ionic strength (M)	treatment	$\bar{M}_w \times 10^{-3}$	% order	$\bar{M}_w/\langle\bar{M}_w\rangle_7$
22	Na^+	Cl^-	0.15	ref 9	145	0	0.93
22	Na^+	Cl^-	0.15	ref 23	163	0	1.04
22	Na^+	I^-	0.15	ref 9	162	100	1.03
						$\langle\bar{M}_w\rangle_7 = (157 \pm 10) \times 10^3$	

f. Data from ref 24 (sample S-1)

T (°C)	counterion	co-ion	ionic strength (M)	$\bar{M}_w \times 10^{-3}$	% order	$\bar{M}_w/\langle\bar{M}_w\rangle_8$
5	K^+	Cl^-	0.10	61	100	1.13
10	K^+	Cl^-	0.10	53	100	0.98
15	K^+	Cl^-	0.10	52	76	0.96
35	K^+	Cl^-	0.10	50	0	0.93
40	K^+	Cl^-	0.20	52	0	0.96

Table 1 (Continued)

f. Data from ref 24 (sample S-1)

T (°C)	counterion	co-ion	ionic strength (M)	$\bar{M}_w \times 10^{-3}$	% order	$\bar{M}_w/\langle\bar{M}_w\rangle_8$
40	K^+	Cl^-	0.20	49	0	0.91
50	K^+	Cl^-	0.30	54	0	1.00
50	K^+	Cl^-	0.30	61	0	1.13
					$\langle\bar{M}_w\rangle_8 = (54 \pm 4) \times 10^3$	

g. Data from ref 24 (Sample S-2)

T (°C)	counterion	co-ion	ionic strength (M)	$\bar{M}_w \times 10^{-3}$	% order	$\bar{M}_w/\langle\bar{M}_w\rangle_9$
5	K^+	Cl^-	0.10	37	100	1.06
10	K^+	Cl^-	0.10	35	100	1.01
15	K^+	Cl^-	0.10	37	76	1.06
35	K^+	Cl^-	0.10	36	0	1.03
40	K^+	Cl^-	0.20	35	0	1.01
40	K^+	Cl^-	0.20	34	0	0.98
50	K^+	Cl^-	0.30	36	0	1.03
50	K^+	Cl^-	0.30	33	0	0.95
					$\langle\bar{M}_w\rangle_9 = (35 \pm 2) \times 10^3$	

only within a somewhat limited range of experimental conditions and that it was very soon followed by a further intermolecular process of chain association. It is obvious to identify such an association process of κ-carrageenan with the previously reported association produced by the gelling K^+ counterion.[11] This point will be more extensively addressed in a following paper.[8]

A series of papers devoted to the investigation of the macromolecular properties of carrageenans appeared soon after.[9,19,20] One of them[9] was devoted in particular to the study of the ion-induced conformational transition of κ-carrageenan by use of WA-TILS, optical activity, and viscosimetry. The accurate determination of both $\bar{M}_w$ and of $\langle R_G^2\rangle_z$ was performed in a parallel series of experiments carried out at 25 °C in aqueous NaCl (disordered form) and in aqueous NaI (ordered form), respectively, as a function of an increasing concentration of the supporting 1:1 electrolyte. The authors devoted particular care to ensure the reproducibility of the results. The preparation of the solutions was given special attention, since it was observed that exposure of freeze-dried κ-carrageenan to high gradients of ionic strength always gave rise to more or less marked association/aggregation effects.[9] The success of such a careful treatment is demonstrated by the linear dependence of the inverse of the reduced scattering intensity, $K\,C_p/R_\theta$, on $\sin^2\theta/2$ over a wide range of angles and by the linear dependence of the same function on the polymer concentration, C_p. The values of the specific optical activity, [α], indicated that from 0.02 to 0.20 M NaCl no change in conformational ordering took place. On the contrary, in the case of NaI, [α] indicated that the disorder-to-order conformational transition fully developed in the molarity range of salt from 0.02 to 0.10 M, afterward remaining constant up to [NaI] = 0.40 M. Well within experimental precision, both the values of $\bar{M}_w$ during the development of the conformational transition and those determined under the condition of 100% ordering (i.e. for 0.10 M ≤ [NaI] ≤ 0.15 M) coincided with those determined for the disordered conformation (Table 1c). That work was the most detailed and convincing support for the conclusions of Grasdalen and Smidsrød that a unimolecular conformational transition of κ-carrageenan is a prerequisite for further chain association.

The light-scattering equipment used in the investigations so far discussed were the "classical" WA instruments, with trailing detector. The values of $\bar{M}_w$ determined always pertained to the unfractionated sample in the conventional TILS mode.

Recently, a paper on the solution properties of κ-carrageenan in the presence of different counterions and co-ions was published by Ciancia et al.,[21] using a variety of techniques. The $\bar{M}_w$ of the polysaccharide, both in its disordered and in its ordered forms, was determined by SEC using a multiangle laser light scattering (SEC-MALLS) apparatus, a capillary viscometer, and a refractometer as serial detectors.[22]

The disordering conditions were achieved both at low temperature (30 °C, using aqueous 0.20 M NaCl as a solvent) and at high temperature (60 °C, in 0.10 M NaI as a solvent). The ordering one corresponded to aqueous 0.10 M NaI at 21 °C. Samples were kept at 50 °C for over 1 h before injection, but first allowed to reach room temperature, so that determinations were made at the indicated temperature (M. Ciancia, private communication).

The $\bar{M}_w$ data of Ciancia et al.[21] have been here reported in Table 1d. They clearly indicate that no doubling of the molecular weight takes place with the full development of the conformational transition, as indicated by parallel optical activity and viscosity data. This result is in full agreement with those obtained by Slootmaekers et al.[9] by static light-scattering determinations in the WA-TILS mode.

A quantitative support to the reliability of those $\bar{M}_w$ values comes from a critical assessment of other data of the paper. The measuring setup used by Ciancia et al.[21] and Tinland et al.[22] is able to provide the value of the intrinsic viscosity, [η], of the polymer under the given chromatographic conditions. For the system being discussed, [η] is 1300 mL g^{-1}, which is even slightly higher than the value reported for the zero-shear intrinsic viscosity of κ-carrageenan in the ordered conformation attained under truly equilibrium conditions (cfr. Table 5 of ref 21). From the above, it must be concluded that during the full transformation of κ-carrageenan from the disordered to the ordered state in aqueous 0.10 M NaI at 21 °C, $\bar{M}_w$ remains constant as expected for an intramolecular conformational transition.

2. Results Obtained Using a LALLS Setup. In all the above papers the inverse reduced light-scattering intensity was extrapolated to zero angle to obtain $\bar{M}_w$. Such a procedure may underestimate the contribution to scattering from even tiny amounts of high molar mass

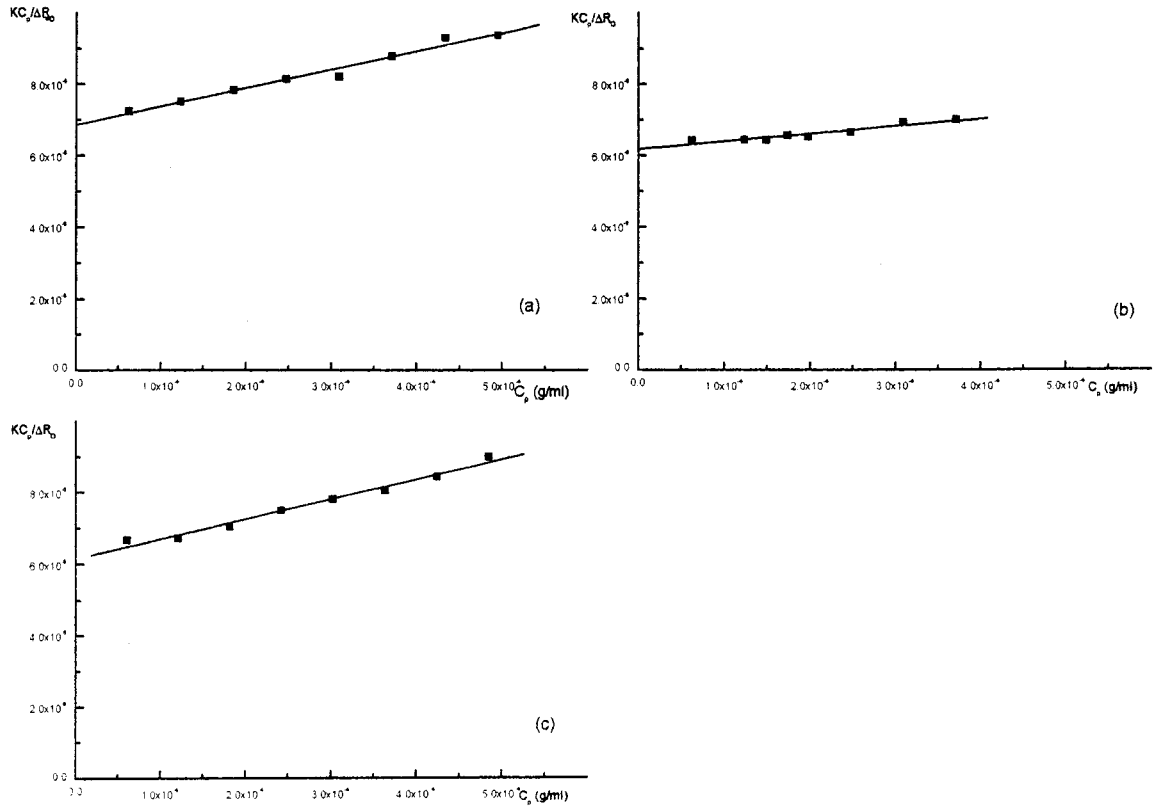

Figure 2. LALLS results of κ-carrageenan in 0.15 M Na^+ halide aqueous solutions, with a linear fitting of the inverse reduced scattering data: (a) solution in NaCl prepared according to the procedure of ref 9; (b) solution in NaI prepared according to the procedure of ref 9; (c) solution in NaCl prepared according to the procedure of ref 23.

material (e.g., aggregates). LALLS apparatuses, on the contrary, allow measurements at very low angles, avoiding the need to resort to extrapolation procedures.

Using LALLS in the TILS mode, a sample with an average molar mass of about 150 000 was chosen intermediate between the different molar mass values reported in previous articles. Also, the temperature (T = 22 °C) was chosen intermediate between the most common value of 25 °C[9] and the lower values used in other studies.[17,18,21]

The ionic conditions producing the limiting cases of the disordered and ordered forms were based on the work of Slootmaekers et al.[9] Aqueous 0.15 M NaCl was used for the former and aqueous 0.15 M NaI for the latter. A value of the molar concentration of iodide of 0.15 had been reported as the highest for which the value of $\bar{M}_w$ was equal for the ordered and the disordered forms, both for Na^+ [9] and TMA^+ [18] counterions. The high sensitivity of the LALLS apparatus used in the TILS mode enabled us to work in a very low range of polymer concentration, C_p (typically from about 6 × 10^{-5} to 5 × 10^{-4} g mL^{-1}). The plot of the inverse reduced scattering intensity vs C_p for the case of 0.15 M NaCl is reported in Figure 2a. It is linear over the whole range of C_p investigated, as is the equivalent plot for 0.15 M NaI (Figure 2b). The relevant parameters of the linear fit of the data are reported in Table 2, from which reliable values of the $\bar{M}_w$ and of the second virial coefficient, A_2, can be calculated. The individual values of the former parameter differ from their mean value by <10%, normally considered to be acceptable for the uncertainty of $\bar{M}_w$ determined by LS methods. As for A_2, the value in iodide solution is remarkably lower than in chloride solution, indicating that iodide is a substantially poorer solvent for the ordered polymer conformation than chloride for the disordered one. This is always found on passing from a semirigid helical conformation to a statistically disordered one. Our A_2 results compare very well with those reported by Slootmaekers et al.[9] under similar conditions.

Table 2. Macromolecular Parameters of κ-Carrageenan in Aqueous Solutions, [Na^+halide-] = 0.15 M at 22 °C, as Determined by LALLS, and linear Fit of the Reduced Inverse Scattering Data as a Function of C_p

co-ion	preparation method according to	correlation coefficient	$\bar{M}_w \times 10^{-3}$	$A_2 \times 10^3$ (mol·mL·g^{-2})	$\chi^2 \times 10^{14}$
Cl^-	ref 9	0.988	146	2.57 ± 0.16	1.70
I^-	ref 9	0.952	162	1.07 ± 0.14	0.57
Cl^-	ref 23	0.991	163	2.80 ± 0.16	1.58

For both of the above cases, the procedure followed for the preparation of solutions was the one most widely accepted for handling potentially associating polyelectrolytes, which carefully avoids exposure of the ionic polysaccharide to strong gradients of electrolyte concentration.[9] For the case of 0.15 M NaCl, the effect on $\bar{M}_w$ produced by another procedure of solution preparation[23] was also investigated. Here the freeze-dried polymer was directly dissolved in an aqueous ionic solution at the desired (comparatively high) electrolyte concentration, followed by stirring at 85 °C for 30 min. The plot of the LALLS data obtained in this way was still linear, and good estimates of the parameters could be obtained. They are reported in the third row of Table 2. The value of $\bar{M}_w$ differs from that obtained using the conventional procedure by <10%, and the two values of A_2 coincide within the limits of standard deviation. That result enables one to conclude that the procedure of ref 23 can still provide reliable values of the macromolecular parameters from LS when the polysaccharide

was freeze-dried in the disordered conformation and that the same *disordered* conformation is expected to prevail in the final conditions (i.e., for the given electrolyte type and concentration). Moreover, this has been demonstrated to hold true only in the very low range of C_p investigated. The three results can then be considered together in Table 1e.

After the above experiments had been performed, an article appeared that supports strongly the reported findings.[24] Using a LALLS detector in conjunction with a SEC setup, Ueda et al.[24] determined the $\bar{M}_w$ of two different samples of κ-carrageenan of moderately low molar mass. By working at different temperature, T, and ionic strength, I, they were able to measure $\bar{M}_w$ over conditions spanning from complete conformational disorder to full chain ordering, as monitored by parallel experiments of optical activity. An important feature of their work was the use of K^+ as a counterion (in aqueous KCl), which is a notorious condition eventually leading to gel formation. At the "low T, low I" end of the conformational variables range, they found that the molecular weight of κ-carrageenan does not change during the full transition from coil to helix. This indicates that "κ-carrageenan ... makes the transition from a coil to a single helical conformation in this (i.e., 0.10 M) KCl salt solution."[24] The corresponding data are reported in Table 1f and g, respectively.

For higher T and I values, Ueda et al.[24] found a progressive increase of $\bar{M}_w$, as expected for an interchain association, which underlies any gel formation; those data will be discussed in the following paper.[8] Besides being timely support for all previous evidence for the intramolecular character of the fundamental conformational transition of κ-carrageenan, those recent data nicely confirm the hypothesis of the existence of a "window" in the conformational phase diagram, in which the fundamental ordered conformation of κ-carrageenan should have been found if looked for,[25] even in the presence of gelling counterions. Microcalorimetric and NMR data, coupled with polyelectrolyte theory calculations,[26] indicated that for the Cs^+ salt form of κ-carrageenan (which shows a behavior remarkably close to that of the K^+ form), such conditions would correspond to temperatures lower than about 10 °C and ionic strength values lower than about 10^{-2} M, fitting perfectly with the findings of Ueda et al.[24]

Concluding Remarks

As a sound conclusion, it can be stated that a very large number of accurate and reliable determinations of M_w have been critically reviewed and others newly obtained for samples of κ-carrageenan of various molar mass values, both in completely disordered conditions and in those of full conformational ordering, as well as in intermediate cases.

From the whole of the above analysis of molar mass data it clearly appears that a wide range of conditions can be found, in dilute aqueous solutions, in which κ-carrageenan is able to undergo the disorder-to-order conformational transition without any change in the average molar mass.

The high number of cases from different laboratories using different polymer samples, the variety of the techniques used, the wide interval of average molar mass investigated, and the reported occurrence both for different counterions (K^+, Na^+, TMA^+) and anions (I^-, Cl^-) are all strong evidence contributing to the universality of the experimental observation. The common feature to all such results is that the conformational transition of κ-carrageenan from the disordered to the fundamental ordered conformation is an *intramolecular* process.

By fundamental ordered conformation it is herewith meant the one that requires the minimum number of chains, or of chain stretches (*cooperative segments*), in the stereochemically ordered structure. In any case, it should be stressed that, under different conditions, the presence of higher levels of ordered stereochemical arrangements cannot be excluded. For the particular case of κ-carrageenan, such tertiary levels of structure must be clearly involved in the chain−chain interactions leading to interchain association and, eventually, to gel formation. The fundamental ordered conformation should be looked upon as just one (albeit the most important from the conformational standpoint) of the various regular conformations often simultaneously present in a real carrageenan system. Under different conditions, further levels of chain association not only can be present, but they even have to be expected, particularly so in the presence of high values of polymer concentration or of ionic strength, or of both.

In any event, the whole of the described findings rules out the possibility that an intertwined (coaxial) double helix be such a fundamental ordered conformation in dilute aqueous solutions. Of course, the same findings cannot rule out the existence of a double helix in the solid state, or, despite a lasting absence of any *direct* evidence, for the aqueous solution-state as a possible higher (tertiary) level of stereochemical order. However, any doubling (more often an alleged doubling or a "quasi-doubling") of $\bar{M}_w$ just reflects the molecularity of the simplest *associated* ordered form and *not* that of the *fundamental* ordered conformation.

The obvious candidate for such a fundamental secondary structure is the single helix, which was originally proposed by Bailey.[6] Such a single helix would more or less exactly correspond to one of the strands of the model double helix, constructed on the basis of X-ray fiber diffraction data,[15] i.e., sharing with it the symmetry (a 3_1 symmetry, or one closely related to it), the pitch, and the repeating-unit advance on the helix axis (about 8.2 Å). By thermodynamic arguments such a helix was repeatedly demonstrated to be the only one compatible with the polyelectrolytic features[25,27] and with the experimental microcalorimetric data in aqueous media[26,28] pertaining both to the intramolecular transition and to the following process of chain pairing. The latter is obviously supposed to be at the origin of the process of chain association in solution and, eventually, of gel formation.

The same combined approach has unequivocally indicated the single helix as the only secondary structure of κ-carrageenan compatible with the calorimetric data both in formamide and in dimethyl sulfoxide (DMSO).[29,30]

Data on molar mass alone are, however, conceptually unable to resolve the problem of the *topology* (e.g., side-by-side or double helical) of the associated form (tertiary level of structure) in solution and in a gel, not to say of the polysaccharide conformation in the solid state (e.g., in a fiber).

Cyclic structures have been reported for some polysaccharides.[31−33] Although of great interest for the understanding of transient structures in highly concen-

trated solutions and in the solid state under very particular conditions of preparation and of measurements, they do not seem to have any bearing on the problem of the fundamental helical conformation of κ-carrageenan in *dilute* and *semidilute* aqueous *solutions*. In fact, such topologies are intrinsically incompatible with, e.g., the very interesting recent report of a novel chiral nematic phase in aqueous κ-carrageenan in the semidilute range of polymer concentration. The cholesteric behavior has been correctly ascribed by the authors to its "rigid, helical secondary structure", which provides close similarity to "rods where the rigidity is provided by a secondary helical structure".[34] The single-helical model then appears to be the obvious choice for such a fundamental helical structure.

Two very recent and independent reports bring additional support to this single-helical model. In a molecular dynamics (MD) solution simulation on two hexasaccharide strands of β-carrageenan (i.e., the uncharged desulfated form of κ-carrageenan) in the double-helical conformation, "the interchain hydrogen bonds did not persist, but rather exchanged for hydrogen bonds to solvent, and possibly as a result, the double helix was observed to begin to unravel. However, the individual glycosidic linkages in the separate strands appear to be more stable in the fiber diffraction conformation in solution than in the vacuum simulation, suggesting that the polysaccharide exists in [aqueous] solution as a single helix which approximates the same conformation as previously proposed for these chains in the double helix".[7]

In a combined NMR and molecular modeling study of κ-carrageenan in DMSO and in water (both with and without the presence of different 1:1 electrolytes), it was recently demonstrated that the polymer already in its so-called disordered conformation is very rigid.[35] The nuclear Overhauser effect (NOE) data provide intra- and intersugar constraints that allow the glycosidic rotational angles to oscillate in a very narrow range around those pertaining to the minimum energy, as indicated both by molecular mechanics and MD calculations. Accordingly, the disordered conformation should be rather indicated as a loose helix (cfr agarose).[36] The set of glycosidic angles for the minimum energy conformation produces a single helix with helical parameters practically coinciding with those proposed on the basis of X-ray diffractograms for the *single filament* of the alleged double helix (see the above results of Ueda et al.[7]). MD calculations predicted the formation of an interresidue hydrogen bond, centered on the sulfate group, the existence of which was successfully demonstrated by the experimentally determined temperature dependence of the proton chemical shift. Such a hydrogen bond is likely to be the key structural element providing for the long-range interresidual stability, as required for explaining the known cooperativity of the disorder-to-order conformational transition. The very high intrinsic rigidity of the loose helix *in aqueous solutions* clearly prevents the polymer from undergoing the intermolecular nucleation and propagation steps necessary for the formation of a double helix. The latter steps normally require a very high intrinsic *flexibility* of the individual filaments, as is well-known in the case of B-DNA. In fact, all molecular modeling attempts at constructing a double helix in accordance with the experimental NOE constraints failed because of overlapping between different parts of the chains.

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 University of Trieste and POLYtech for hospitality in their laboratories.

References and Notes

(1) Painter, T. J. Algal Polysaccharides. In *The Polysaccharides*; Aspinall, G. O., Ed.; Academic Press: New York, 1983; Vol. 2, p 196.

(2) Rees D. A.; Steele, I. W.; Williamson, F. B. *J. Polym. Sci. Part C* **1969**, *28*, 261−276.

(3) Morris, E. R.; Rees, D. A.; Robinson, G. *J. Mol. Biol.* **1980**, *138*, 349−362.

(4) Nilsson, S.; Picullel, L. *Macromolecules* **1989**, *23*, 3011−3017.

(5) Nerdal, W.; Haugen, F.; Knutsen, F.; Grasdalen, H. *J. Biomol. Struct. Dyn.* **1993**, *10*, 785−791.

(6) Bailey, S. T. *Biochim. Biophys Acta* **1955**, *17*, 194−205.

(7) Ueda, K.; Imamura, A.; Brady, J. W. *J. Phys. Chem.* **1998**, *102*, 2749−2758.

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

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

(10) Martinsen, A.; Skjåk-Braek, G.; Smidsrød, O.; Zanetti, F.; Paoletti, S. *Carbohydr. Polym.* **1991**, *15*, 171−193.

(11) Snoeren, T. H. M.; Payens T. A. J. *Biochim. Biophys. Acta*, **1976**, *437*, 264−272.

(12) Robinson, G.; Morris, E. R.; Rees D. A. *J. Chem. Soc. Chem. Commun.* **1980**, 152−153.

(13) Smidsrød, O. In *27th Int. Congress of Pure and Appl. Chem.*; Varmavuori, A., Ed., Pergamon: Oxford, 1980; pp 315−327.

(14) Smidsrød, O.; Andresen, I.-L.; Grasdalen, H.; Larsen B.; Painter, T. *Carbohydr. Res.* **1980**, *80*, c11−c16.

(15) Anderson, N. S.; Campbell, J. W.; Harding, M. M.; Rees, D. A.; Samuel, J. W. B. *J. Mol. Biol.* **1969**, *45*, 85−99.

(16) Grasdalen, H.; Smidsrød, O. *Macromolecules* **1981**, *14*, 1842−1845.

(17) Smidsrød, O.; Grasdalen, H. *Hydrobiologia* **1984**, *116/117*, 19−28.

(18) Smidsrød, O.; Grasdalen, H. *Hydrobiologia* **1984**, *116/117*, 178−186.

(19) Slootmaekers, D.; Mandel, M.; Reynaers, H. *Int. J. Biol. Macromol.* **1991**, *13*, 17−25.

(20) Slootmaekers, D.; van Dijk, J. A. P. P.; Varkevisser, F. A.; Bloys van Treslong, C. J.; Reynaers, H. *Biophys. Chem.* **1991**, *41*, 51−59.

(21) Ciancia, M.; Milas, M.; Rinaudo, M. *Int. J. Biol. Macromol.* **1997**, *20*, 35−41.

(22) Tinland, B.; Mazet, J.; Rinaudo, M. *Makromol. Chem. Rapid Comm.* **1988**, *9*, 69−73.

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

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

(25) Paoletti, S.; Smidsrød, O.; Grasdalen, H. *Biopolymers* **1984**, *23*, 1771−1794.

(26) Paoletti, S.; Delben, F.; Cesàro, A.; Grasdalen, H. *Macromolecules* **1985**, *8*, 1834−1841.

(27) Benegas, J. C.; Cesàro, A.; Rizzo, R.; Paoletti S.; *Biopolymers* **1998**, *45*, 203-216.

(28) Cesàro, A.; Delben, F.; Paoletti, S.; Scagnolari, F. *Thermochim. Acta* **1985**, *85*, 465−468.

(29) Rochas, C.; Rinaudo, M. *Carbohydr. Res.* **1982**, *105*, 227−236.

(30) Benegas, J. C.; Pantano, S.; Vetere, A.; Paoletti, S. *Biopolymers*, in press.

(31) Stokke, B. T.; Elgsaeter, A.; Kitamura, S. *Int. J. Biol. Macromol.* **1993**, *18*, 223−229.

(32) Abeysekera, R. M.; Bergström, E. T.; Goodall, D. M.; Norton, I. T.; Robards, A. W. *Carbohydr. Res.* **1993**, *248*, 225−231.

(33) McIntyre, T. M.; Brant, D. A. *Biopolymers* **1997**, *39*, 133−146.

(34) Borgström, J.; Quist, P.-O.; Piculell, L. *Macromolecules* **1996**, *29*, 5926−5933.

(35) Bosco, M.; Segre, A. L.; Miertus, S.; Paoletti, S. In *Proceedings of the Italian Macromolecular Society Meeting, AIM*, Genoa-,September 1997; pp 688−690.

(36) Rochas, C.; Brûlet, A.; Guenet, J. M. *Macromolecules* **1994**, *27*, 3830−3835.

MA981203Z