Macromolecules 1994, 27, 4160–4166

On the Mechanism of Gelation of Helix-Forming Biopolymers

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Received January 25, 1994[⊗]

ABSTRACT: The theory of double-helix formation has been applied to experimental coil–helix transition curves published by Rochas *et al.* (*Carbohydr. Polym.* **1990**, *12*, 2551) on κ-carrageenan samples covering a large range of molecular weights. Within the uncertainty due to sample polydispersity, the theory satisfactorily reproduces the experimental data, yielding values for the microscopic parameters (the association and initiation parameters) that govern the cooperativity of the transition. These parameters were used in a detailed theoretical analysis of the helix formation of κ-carrageenan, which indicates that (1) an all-or-none transition is a poor description of the process, (2) the concentration dependence of the transition is significant only for the short chains, and (3) the double-helical regions are always too few to generate an infinite network by helix formation alone. The analysis implies that the κ-carrageenan gel network is *not* created on the helical level, by each chain forming double-helical junctions with, on the average, two or more partners. The latter conclusion was supported by a viscosimetric experiment, performed under nonaggregating conditions (in 0.2 M LiI), which showed that the relative viscosity of a κ-carrageenan solution was a monotonic function of the helical content in the system. A further experiment showed that a nongelling solution of helical rods of κ-carrageenan could be reversibly transformed into a gel by dialysis against an appropriate salt solution under such conditions that the carrageenan molecules never pass the coil state. It is concluded that the κ-carrageenan gel network is created essentially on the superhelical level, by helix aggregation.

I. Introduction

Many biopolymers with the ability to form a gel in aqueous solution,[1] e.g. agarose, carrageenan, and gelatin, have one essential feature in common, i.e. that the gelation is preceded by a transition from a disordered state (random coil) to an ordered conformation (helix) according to the following scheme.

$$\text{coil} \rightarrow \text{helix} \rightarrow \text{gel} \qquad (1)$$

The helical state may be induced by lowering the temperature, by changing the solvent, or by adding salt.[2] The occurrence of gelation in certain systems implies that the helix formation, in these systems, leads to an *association* between the polymer strands. However, for gelation to occur, the association must also include *branching*, so that an infinite three-dimensional network is formed. Figure 1 shows, schematically, two possible mechanisms for association and branching where the helical state, in one way or another, is involved. In Figure 1a the association and branching occur on the *helical* level, through incomplete formation of multiple helices such that each active network chain joins in helices with two or more other chains. This is the classical picture of gelation through helix formation, which is the currently accepted model for gelatin,[3] but which has also frequently been invoked for the helix-forming gelling polysaccharides. Figure 1b, on the other hand, represents a network formation on the *superhelical* level, where fully developed (single or multiple) helices aggregate to form a gel. This type of mechanism has been suggested[4] for the synthetic polypeptide poly(γ-benzyl α,L-glutamate) in benzyl alcohol. More complex mechanisms, involving both branching on the helical level and superhelical aggregation, have also been proposed. One such model is the domain model[5] for carrageenans, according to which finite network domains are created by branching on the helical level, followed by the aggregation of the domains on the superhelical level into an infinite network. The branching on the helical level is considered to be an essential feature also in the domain model.

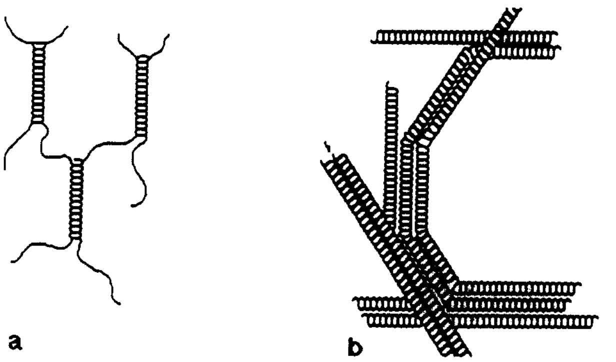

Figure 1. Schematic drawings of possible mechanisms of network formation in helical gels: gelation on the helical (a) and superhelical (b) levels.

Model calculations relevant to the formation of an equilibrium network of the helical level have been presented by Higgs and Ball.[6] They modified the Zimm–Bragg[7] model for a linear chain to represent a branched equilibrium network, formed by pairwise bonding of the chain molecules. Their analysis shows that the fraction of bonded repeating units is a decreasing function of temperature, while the number of bonded sequences per molecule passes through a maximum at some intermediate temperature. For sufficiently long polymers, given a finite cooperativity of the pair formation, this maximum number of bonded sequences exceeds the Flory–Stockmayer gelation point (where the mean number of bonded sequences per chain is 2), and an infinite network is formed. As the temperature is decreased below the temperature of the maximum functionality, the network eventually breaks up again, owing to the merging of the bonded sequences.

The analysis of Higgs and Ball underlines the importance of detailed information on lengths and distributions of the helical regions on the polymer, under varying conditions of helical content, for the molecular understanding of the gelation mechanism of any real biopolymer. The coil–helix transition theory[8] provides the possibility to

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[⊗] Abstract published in *Advance ACS Abstracts*, June 15, 1994.

0024-9297/94/2227-4160$04.50/0

obtain such information, provided that experimental data on the molecular-weight dependence of the transition is available. Such data are not readily obtained, however, and detailed analyses of conformational transitions of polysaccharides are correspondingly scarce.[9,10] Furthermore, it is important to establish experimental procedures whereby helix formation and helix aggregation may be studied separately. With the normal cold-setting procedure, helix formaion, helix aggregation, and gelation typically occur simultaneously, making it very difficult to decide on which level the necessary network branching is produced.

Both the above routes are being pursued here for κ-carrageenan, a linear sulfated polysaccharide with a backbone based on a repeating disaccharide unit of D-galactose and 3,6-anhydro-D-galactose, which may be extracted from many species of red seaweeds.[11] κ-Carrageenan is well-known for its gel forming properties and is used extensively in the food industry as a gelling agent. κ-Carrageenan is particularly well suited for our purposes, since detailed experimental data on the molecular-weight dependence of its conformational transition are available, and since it is possible to vary the extent of helix aggregation by the choice of salt. A detailed study[12] by Rochas, Rinaudo, and Landry (RRL) describes the temperature dependence of the optical rotation for a series of κ-carrageenan fractions, prepared by ultrasonication, preparative gel permeation chromatography, or hydrolysis, which cover an exceptionally large molecular weight range (from 6.6×10^3 to 1.2×10^6). Although there has been some controversy regarding the multiplicity of the κ-carrageenan helix, it is by now firmly established that the ordered conformation is a double helix.[11,13] The most troublesome evidence to the contrary comes from studies that have failed to show the expected doubling of the molecular weight on helix formation.[14,15] However, recent studies by multiangle laser light scattering performed in our laboratory on a range of κ-carrageenan fractions did indeed show the expected doubling of the molecular weight.[16] It is unclear why a change of the molecular weight is not always seen, but intramolecular helix formation[17] through cyclization or hairpin formation at high dilution is a possible explanation.

A substantial aggregation of the κ-carrageenan helices occurs under the ionic conditions (presence of certain cations, such as potassium, rubidium, or cesium) normally used in order to achieve gelation.[13,18-20] However, it has also been shown that certain anions, such as iodide and thiocyanate, prevent helix aggregation while still increasing the stability of the helix itself.[21,22] A number of studies from our laboratory have shown that both the specific anion and the specific cation effects on the helix stability may be explained by site-binding of the relevant ions to the κ-carrageenan helix.[23-26] Various models[5,20,27-33] have been suggested for the gelation of κ-carrageenan. Most of these focus on the nature and origin of the intermolecular association between the κ-carrageenan molecules, rather than on the origin of branching and network formation. Notable exceptions are the models suggested by Rees and co-workers,[5,28] according to which a branching on the helical level, caused by the presence of helix-incompatible "kink" units in the carrageenan chains, is an essential feature.

This paper is organized as follows. First, we briefly review the relevant coil–helix transition theory, which we then apply to the experimental data of RRL. Then we use the model parameters thus extracted to perform an analysis of the lengths and distributions of helical regions, as well as the concentration dependence of the coil–helix transition, for κ-carrageenan chains of various degrees of polymerization. Finally, we present some relevant experimental results of our own, including a study of the temperature dependence of the viscosity of κ-carrageenan solutions across the coil–helix transition under nonaggregating conditions, and a study of a reversible, isothermal gelation of helical κ-carrageenan by dialysis against appropriate salt solutions. A brief account of this work has been presented previously.[34]

II. Experimental Section

Materials. Commercial samples of κ-carrageenan (from *Euchema cottonii*) were obtained from Sigma Chemicals Co. The samples were purified and ion exchanged to the desired pure ion forms, as described previously.[35]

Methods. The viscosity was measured with an Ostwald viscosimeter immersed in a thermally regulated water bath. The dialysis experiments were performed with Spectrapor dialysis tubes with a molecular weight cutoff of 6–8000.

III. Theory of Coil–Helix Transitions

The theoretical modeling of coil–helix transitions is thoroughly described elsewhere.[8] Here we only give a short review of the main features and assumptions of the theory, as applied to the formation of a double helix involving two strands of a homopolymer.

To account for the fact that the double-helix formation is a bimolecular reaction, the model first considers the process

$$2\mathrm{C} \underset{\beta s}{\leftrightarrow} \mathrm{H}_1 \tag{2}$$

where a bimolecular aggregate, H_1, containing one repeating unit of a double helix, is formed from two randomly coiled single strand molecules, C, each containing N repeating units. The equilibrium constant for this process is βs, where s is the stability constant for helix formation (cf. below) and β is the so-called association factor, representing additional entropy changes associated specifically with the formation of the first repeating unit of the double helix. The dimensionless product $\beta[\mathrm{C}]$, where [C] is the concentration of coil molecules, also includes the loss in translational entropy associated with the initiation of a helix from two independent molecules. This product is generally much smaller than unity and accounts for the intrinsic cooperativity of the double-helix formation.

The next process to be considered is the helix propagation

$$\mathrm{H}_n \underset{s}{\leftrightarrow} \mathrm{H}_{n+1} \tag{3}$$

Here H_n represents a species with n consecutive bonded repeating units. This step represents the successive adding of units to the double helix. Since the model assumes that there are only nearest-neighbor interactions between the repeating units of the polymer chain, the equilibrium constant for this process is taken to be equal to s for all values of n in the interval $[1, N-1]$. It is usually assumed that s contains all enthalpic contributions to the helix formation; i.e. the possibility is neglected that the enthalpy of forming the first helical unit in a helical section may be different.

Finally, there exists the possibility that a double strand may join in more than one double-helical region, separated by loops of disordered units. A loop of i residues is assigned a special statistical weight, the loop correction factor, which has the form of σi^{1-c} when the two chains forming the loop are allowed to be unequal in length. The factor i^{1-c}

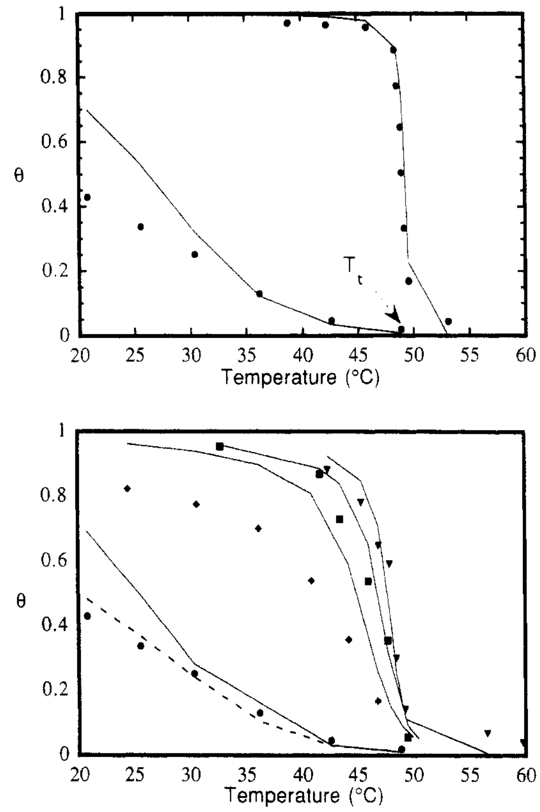

Figure 2. (a, top) Theoretical fits (lines) to experimental[12] coil–helix transition data (points) used to obtain (see text) the association and initiation parameters for κ-carrageenan. (b, bottom) Experimental[12] (points) and theoretical (solid lines) coil–helix transitions of κ-carrageenan fractions with average numbers of repeating disaccharides of, from left to right, 16, 78, 142, and 247. The dashed line represents the curve for a polydispersity index of 1.15, N_w = 16 (see text).

accounts for the fact that the entropy loss associated with the loop closure (the merging of two chains to form a new helical section) depends on the size of the loop.[36,37] The exponent c has the value 1.75 when allowance has been made for the excluded volume effect,[38] i.e. the exclusion of conformations where two parts of the chain occupy the same space at the same time. The initiation parameter σ, finally, accounts for additional entropy changes associated with the formation of the first repeating unit of the new double-helical region.

IV. Model Calculations and Parameters

The partition function that describes the coil–helix transition for our system is a function of the parameters N, s, σ, and β. These parameters were optimized so as to give the best possible theoretical reproduction of the experimental data of RRL (Figure 2) by the following procedure. The degree of polymerization, N, was taken as equal to the weight average molecular weight, as given by RRL, divided by 408.3, which is the molecular weight of the repeating disaccharide (Na form) of κ-carrageenan. The fraction of helical residues, θ, from experiment was obtained from specific optical rotation data, $[\alpha]$, at 365 nm using the relation

$$\theta = ([\alpha] - [\alpha]_c)/([\alpha]_h - [\alpha]_c) \qquad (4)$$

where $[\alpha]_h$ (=271) and $[\alpha]_c$ (=164) are the optical rotation values for all-coil and all-helix samples, respectively, obtained from the low- and high-temperature data for the highest molecular weight of Figure 2 of RRL. Optical rotation data from other samples of RRL were normalized by setting the baseline for the all-coil state to the same value for all carrageenan fractions.

Table 1. Parameters Used in Calculations of Coil–Helix Equilibria

β (1/M)	ΔH (kJ/mol)	T_t (K)	σ
0.19	−21	322	2.6×10^{-4}

The (temperature-dependent) stability constant s was assumed to be given by[39]

$$\ln s = (\Delta S/R) - (\Delta H/RT) \qquad (5)$$

where ΔS and ΔH are the (temperature-independent) entropy and enthalpy, respectively, for reaction 3, in units per mole of disaccharide units. In principle, ΔH could be obtained from calorimetric measurements, as the parameters β and σ are assumed not to contain any enthalpic contributions. In practice, however, the large scatter in the calorimetric transition enthalpy values published for κ-carrageenan (3.5–24.5 kJ/mol, depending on system conditions and experimental procedure[18,22,40–46]) presents a problem. As an estimate, we have here chosen to use the value ΔH = 21 kJ/mol, as obtained previously[24] by two of us in a thermodynamic analysis of the coil–helix transition of κ-carrageenan, where also the contribution from specific counterion binding was included. (The calorimetric transition enthalpy depends essentially on the nonelectrostatic transition enthalpy and on the degree of site-binding of the ions to the helix.) The uncertainty inherent in this approximate procedure should not affect the conclusions drawn here.

To obtain a numerical value of the transition entropy, we observe that s = 1 at some temperature T_t and hence, from eq 5

$$\Delta S = \Delta H/T_t \qquad (6)$$

We have obtained T_t from the midpoint of the coil–helix transition for the highest molecular weight (N = 3000) in the experimental data of RRL (Figure 2a), where it is clear (cf. Figure 2 of RRL) that the molecular-weight dependence of the coil–helix transition has leveled off. Other choices of T_t have been suggested,[8,47] but, in light of the sharpness of the transition considered, these would give practically the same result.

The association and initiation parameters were evaluated separately, by fitting the theory to data for very short chains (where the occurrence of loops may be neglected) and for very long chains (where the occurrence of free chain ends may be neglected), respectively.[8] Accordingly, we evalulated β by applying the staggering zipper model[47] to the experimental data for the shortest chain investigated by RRL, with N = 16 (Figure 2a). In this limiting model, only one helical sequence is allowed for each molecule. This is based on the assumption that it costs too much free energy to make an interruption in the helical sequence of a short chain. The model does, however, allow the strands to be staggered, i.e. the ends do not have to be in phase with each other, since all monomers are identical.

To obtain a numerical value of σ, on the other hand, we used the limit of an infinite chain, and applied the infinite imperfect matching model[8] to the experimental data for the highest molecular weight available (N = 3000; cf. Figure 2a). Here it is assumed that the strands are infinitely long, so that any coil regions must be in the interior, rather than at the ends, of the double strand. The term imperfect has the same meaning as staggering had above.

The four parameters thus obtained are summarized in Table 1. The low value of σ reflects the high cooperativity of the transition (cf. the sharpness of the right transition in Figure 2a) and is comparable to values previously obtained for DNA.[8] The value of β, on the other hand, is higher than the range of values obtained for oligoadenylic acid by Applequist and Damle.[47] The uncertainties in the fitted values of σ and β are difficult to evaluate, but from trial calculations where the effect on the transition of changing these parameters was checked, we regard the values quoted in Table 1 as order-of-magnitude estimates.

In all calculations given below, we have used the parameters of Table 1 together with the partition function for intermediate[8] chains. In these calculations, the number of helical regions was limited to 2 and the number of loops to 1. This limitation of the partition sum should not affect the qualitative conclusions drawn below, since the calculations showed that the second term was always small compared to the first. Thus, in the worst case, i.e. at a helical content of roughly 50% for the longest chain considered (247 repeating units), the maximum contribution for the term corresponding to a second helical region only amounted to 11% of the partition sum.

V. Test of the Model

To test the model, we compared model calculations with experimental data of RRL on transitions of intermediate chain lengths. The results, given in Figure 2b, show that the theory gives a semiquantitative description of the transition for the range of molecular weights studied. The only parameter that is varied in the calculations is the (known) value of N. The concentration of polymer *molecules*, needed for the evaluation of the equilibrium (2), was obtained as the total concentration of repeating disaccharide units (which was kept constant at 1.3 mM for all samples in the experiment) divided by N.

An obvious source of the discrepancies between experiment and model predictions is the polydispersity of the real samples, which will broaden the transitions. To obtain a rough estimate of this effect, we made a simple model calculation for the $N = 16$ chain, assuming a rectangular distribution in molecular weights between $N = 5$ and $N = 23$. This distribution has the desired weight average degree of polymerization of $\langle N \rangle_w = 16$, and a polydispersity index of $\langle N \rangle_w / \langle N \rangle_n = 1.15$, which is lower than the typical values given by RRL for their fractionated samples. (Polydispersity data were, unfortunately, not given for all samples.) The calculated transition curve for the polydisperse mixture was obtained simply as a weighted sum of contributions from monodisperse fractions in the assumed interval, where the calculation for each fraction was done at the nominal concentration of 1.3 mM of repeating units. (This is obviously an approximation, since no pairing between chains of differing degrees of polymerization is considered. Allowing for the latter possibility would, however, make the calculations prohibitively complicated.) The result from this calculation is also seen in Figure 2b, which shows that this estimate reproduces the experimental points quite well. From this we conclude that polydispersity is likely to be the main source of the discrepancy between model and experiment in Figure 2b. The worst fit in Figure 2b is for the chain with $N = 78$. Here, however, the experimental curve at low temperatures seems to level off at a suspiciously low helical content, suggesting the presence of a significant amount of low-molecular-weight material in this particular sample, which was obtained by hydrolysis.

The data of RRL were obtained for κ-carrageenan samples at a concentration of 0.5 g/L in 0.1 M KCl. Under these salt conditions, the helices are expected to aggregate which, in principle, means that the equilibrium treatment applied above is incorrect. However, the aggregation should only give rise to a minor perturbation of the coil–helix transition curves compared to the true equilibrium curves, since aggregation-driven changes in the optical rotation of κ-carrageenan in KCl are known occur on a very slow time scale.[48] A further potential complication in the analysis is the assumption of only nearest-neighbor interactions, as each repeating unit of κ-carrageenan contains a charged sulfate group, which leads to long-range electrostatic interactions. In 0.1 M salt, however, the Debye length is of the order of 1 nm, and the electrostatic interactions are effectively screened.

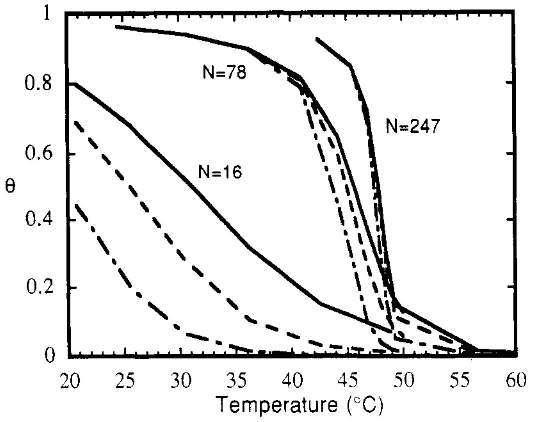

Figure 3. Theoretical coil–helix transition of κ-carrageenan fractions with, solid lines from left to right, 16, 78, and 247 repeating disaccharide units, at total concentrations of 13 (solid), 1.3 (dashed), and 0.13 (dot-dashed) mM.

VI. Model Predictions

Having confirmed the ability of the model to reproduce the experimental data of Figure 2, we used the model parameters of Table 1 to theoretically investigate various features of the helix formation that are difficult to extract from experiment. Figure 3 shows how the transition varies with polymer concentration for different chain lengths. The concentration range covers two decades in concentration and refers to dilute experimental conditions. It is seen that the concentration dependence is only significant for short-chain polymers and is rapidly becoming negligible for increasing chain lengths. These findings demonstrate that a concentration invariance of the transition of long chains, as has been observed for κ-carrageenan,[14] is indeed consistent with a cooperative bimolecular reaction—despite previous assertions to the contrary.[14] Attempts to experimentally confirm the concentration dependence that is predicted for *short* chains were not successful. Optical rotation measurements were performed on fragmented[50] κ-carrageenan chains dissolved in 0.3 M KCl in the concentration range 2.5–20 g/L, but the uncertainty in the data, which was large especially at the lower concentrations, was found to be larger than the expected concentration dependence.

Figure 4 shows how the number average length of a helical section, $\langle L_H \rangle$, changes through the coil–helix transition as the fraction of helical units varies from zero to unity. The helical length is seen to be a smooth function of the total helical content, implying that an all-or-none transition, as has been proposed for ι-carrageenan,[13,51] is a poor description of the transition of κ-carrageenan. (An all-or-none transition assumes that the polymer exists either in the coil state or in the helix state and that intermediate conformations may be neglected.) We suspect—although this point remains to be proven—that

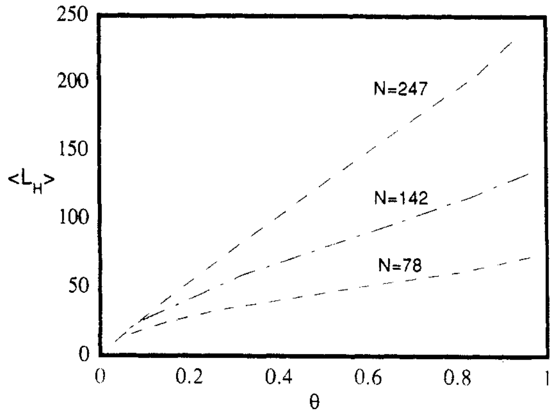

Figure 4. Model predictions of the average number of disaccharide units in the helical regions, as a function of the overall degree of helix conversion, for carrageenan fractions with 78, 142, and 247 repeating units.

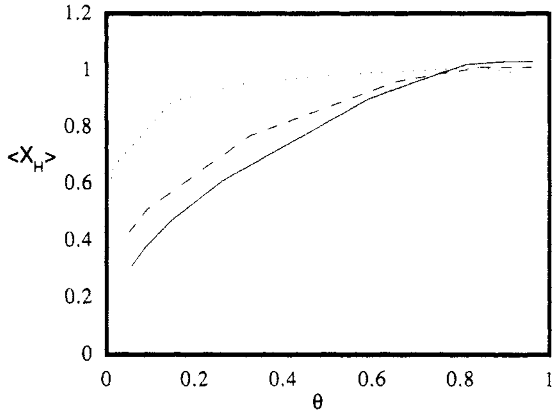

Figure 5. Model prediction of the average number of helical sequences on the double strand, as a function of the total helical fraction, for carrageenan fractions with 78 (solid), 142 (dashed), and 247 (dotted) repeating units.

this conclusion is valid also for ι-carrageenan, as available experimental data indicate that the transition for this carrageenan is *less* sharp (less cooperative) than that of κ-carrageenan.

The frequent occurrence of short helical sequences on the double strand might be taken to suggest that a gelation mechanism according to Figure 1a, the helical mechanism, would be possible. However, a network formation on this level requires that there are at least, on the average, two helical sequences on the chain.[6] Figure 5 shows the calculated average number of helical sections, $<x_H>$, for different chain lengths. As expected, this number increases with increasing chain length, but for the range of polymers investigated (corresponding to a weight average molecular weight up to 10^5), it is always less than 1. This means that the system is far from the Flory–Stockmayer gelation point. The slowness in the increase of the number of helical sections is due to the cooperativity of the transition; to a large extent, an increase in the helical content occurs through a growth of the existing helical regions, rather than through the initiation of new helices. Clearly, for very long chains, a network formation on the helical level should still take place. RRL showed, however, that the molecular-weight dependence of the gel modulus for κ-carrageenan has very nearly leveled off at a molecular weight of 10^5. Thus, the real system has reached its plateau gel structure already at a molecular weight when our calculations indicate an insignificant cross-linking on the helical level.

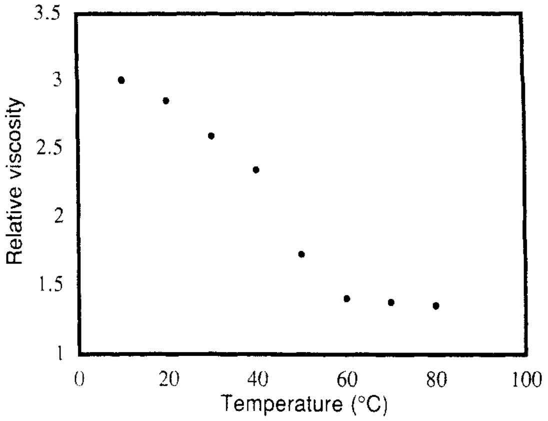

Figure 6. Relative viscosity of 0.1% κ-carrageenan in 0.2 M LiI as a function of temperature.

VII. Experimental Investigations of Network Formation

The theoretical analysis in the preceding section suggests that there is insignificant network formation on the helical level for κ-carrageenan. By implication, the κ-carrageenan gel network must then be created on the superhelical level. Two simple experiments were performed to test these predictions. They both utilize the fact that it is possible to make nongelling solutions of helical κ-carrageenan in iodide salt solutions.[14,21,22] An all-helical solution is not expected to show any network formation, and indeed, light scattering experiments have consistently failed to detect larger polymolecular aggregates of all-helical κ-carrageenan in iodide solutions.[15,16] At intermediate helical contents, however, a network may still be formed, as suggested in the analysis of Higgs and Ball, as a result of cross-linking on the helical level. To test this possibility, we simply measured the viscosity of a solution of κ-carrageenan in LiI through the coil–helix transition (Figure 6). The relative viscosity is seen to be a monotonically growing function of the helical content (temperature) in the system, whereas the occurrence of a network (or, indeed, a substantial fraction of large, but finite, aggregates) at intermediate helical contents would be expected to give rise to a maximum in the viscosity. The absence of indications of network formation on the helical level is clearly consistent with our theoretical analysis.

To further investigate the origin of network formation of κ-carrageenan, we devised a scheme to separate the helix formation and helix aggregation processes. This is important for the following reason: A normal cold-set gel is formed in the presence of a salt that induces aggregation, and the helix aggregation should commence already before the helix conversion is complete. It could then be imagined that, if polymolecular aggregates are formed at an intermediate degree of helix conversion by branching on the helical level, such aggregates would be "frozen in" by subsequent helix aggregation, thus preventing the molecules involved in the aggregates to convert, at lower temperatures, into the thermodynamically favored state of perfect double helices. This mechanism, which may be regarded as a variety of the domain model, would thus yield a network by aggregation of structures that are branched on the helical level.

To separate helix formation and aggregation, we first prepared a nongelling 0.5% solution of all-helical κ-carrageenan in a 0.3 M LiI solution. This solution was subsequently dialyzed against 0.3 M KCl; i.e. it was exposed to salt conditions that induce aggregation. (With a normal cold-setting procedure, 0.5% κ-carrageenan in 0.3 M KCl gives a strong gel.) We found that a gel, visibly indistinguishable from a cold-set gel, was formed also by the dialysis procedure. (Comparisons of the rheology of cold-set and salt-set gels will be the subject of a forthcoming study.) It should be noted that the dialysis conditions were such that the coil content should have been insignificant at all stages. This conclusion is based on studies of the helix formation of κ-carrageenan in solutions of mixed salts of a gelling cation and a helix-inducing anion, which showed that the helix is, in fact, more stable in the mixed salts at a constant ionic strength.[25] The salt-induced gelation was also found to be reversible; dialysis of the gel against 0.3 M LiI gave a clear solution again. A similar dissolution in LiI was also found for a cold-set gel under the same salt conditions. This is a strong indication that branching on the helical level is not required for the gelation of κ-carrageenan and that the network, whether cold-set from initially randomly coiled molecules or salt-set from helical molecules, is essentially formed on the superhelical level.

VIII. Concluding Discussion

As far as we know, the data analyzed here provide the largest molecular-weight range ever subjected to an analysis using the coil–helix transition theory. No other system seems to have been analyzed which spans the entire range from the short chain (staggering zipper) to the infinite (incomplete matching) chain behavior. It is of some fundamental interest that all these data can be rationalized consistently within a single theoretical framework. As regards the issue central to this investigation, i.e. the gel formation of helical polymers, it is, furthermore, evident that a detailed analysis of the coil–helix transition provides valuable molecular insight. Lengths and distributions of helical sequences are fundamental parameters in this context. For the particular case of κ-carrageenan, the analysis performed here provides new answers to many questions raised in the past. The absence of a concentration dependence is shown to be consistent with a cooperative double-helix formation, and for such a cooperative process, in the relevant molecular-weight range, the helical regions are seen to be too few to generate significant cross-linking on the helical level. Still, the transition is not infinitely cooperative, and an all-or-none model seems to be a poor assumption.

For κ-carrageenan, both calculations and experiment point to a gelation on the superhelical level. Particularly significant in this context is the finding that a gel may be produced isothermally from a helical solution, simply by modifying the helix–helix interaction by salt. To the best of our knowledge, this is the first time that the coil-to-helix and helix-to-gel steps in the gelation of a red-algal polysaccharide sample have been separated experimentally.

Nowhere in this study did we find any evidence for branching of the helix caused by kinks in the carrageenan chain. Studies on native and alkali-treated ι-carrageenan[49] in our laboratory have shown that the conformational transition of the alkali-treated sample, where helix-incompatible precursor[50] units have been converted into helix-compatible units, is much sharper than the transition of an untreated sample. This shows that kinks, when present in the carrageenan chain, indeed have the expected effect of lowering the cooperativity of the transition. It is likely that, for an untreated sample, the cooperativity is governed by the lengths of the regular sequences of helix-forming units, rather than by the overall molecular weight. Yet, for the (alkali-treated) κ-carrageenan samples studied by RRL, the sharpness of the transition still increased with increasing molecular weight up to a few hundred thousand. This indicates that kinks do not affect the transition, either because they are too few or because they are, in fact, forced into the helical structure. The same conclusion may be drawn from our investigations of κ-carrageenan in iodide solutions, where we have not seen any evidence, either from viscosity or from light scattering,[16] of the formation of branched structures.

It may be expected that a number of helical polysaccharides, whose gelation follows a scheme very similar to that of κ-carrageenan, also form gels by the same mechanism, i.e. by the formation of a branched network on the superhelical level. This includes not only the chemically related polysaccharides from red algae (agarose, furcellaran, and ι-carrageenan)[1] but also certain gelling microbial polysaccharides, such as gellan gum.[52,53] Still, it is not clear why the helix aggregation, in the gelling systems, produces a sample-filling network, and not simply a precipitate. To gain more insight into these questions, we are currently studying, in our laboratory, the organization of helical solutions and of the salt-induced aggregates of κ-carrageenan.

Acknowledgment. L.P. acknowledges inspiring discussions on the subject of gelation with Madeleine Djabourov. This work was supported by grants from the Swedish Natural Science Research Council and from The Swedish National Board for Industrial Technical Development.

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