The Helix–Coil Transition of an Ionic Polysaccharide Probed by Counterion Self-Diffusion Measurements

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ABSTRACT: The temperature-induced helix–coil transition of the ionic polysaccharide ι-carrageenan in salt-free aqueous solution is studied by monitoring the self-diffusion of the counterion, tetramethylammonium, by the Fourier transform NMR pulsed-gradient spin–echo technique. The results are analyzed within the model developed by Yoshida (*J. Chem. Phys.* **1978**, *69*, 4867), which predicts the steady-state radial diffusion of counterions in a cell containing a cylindrical rod of uniform charge density from the solution of the Poisson–Boltzmann equation in cylindrical symmetry. The choice of rod radii in modeling real polyions undergoing conformational transitions is discussed. Good agreement between experiment and theory is obtained if the lateral counterion diffusion coefficient in the cell is treated as a free parameter, being reduced by the same amount for both helix and coil conformations. It is found that the method is able to distinguish between different proposals for the helix conformation in solution, and the evidence presented rules out the possibility that the helix, under the conditions studied, is a single helix with a solution conformation corresponding to one of the strands of the solid-state double helix.

I. Introduction

A phenomenon common to many regular polynucleotides, polypeptides, and polysaccharides is their cooperative transition, in solution, from a randomly coiled conformation with considerable freedom of reorientation around the inter-residue bonds to a helix conformation, characterized by bond rotational angles that are fixed within very narrow limits. The helix conformation, being much less flexible, is inherently less soluble than the coil, and therefore, the coil-to-helix transition is often followed by aggregation or phase separation. However, in the case of ionic polymers, also the helix may be stable in solution, owing to repulsive polyion–polyion interactions of electrostatic origin, and it is a fact that polymers that may undergo the entire coil–helix transition *in solution* are often polyions. In such systems, the coil–helix equilibrium is influenced not only by temperature but also by the amount and type of added electrolyte.

Information on the interactions between the small ions and the polyion (in its two different conformations) is desirable for at least two reasons: First, an understanding of these electrostatic interactions is a prerequisite for the understanding of the effects of salt and polyelectrolyte concentration on the coil–helix equilibrium. Second, owing to the fact that the distance between consecutive charged groups of the polyion depends on its conformation, information on the conformations of the polyion in solution may be gained, via a suitable electrostatic model, from measurements of the polyion–counterion interactions. The conformational information thus gained is usually obtained as the projected charge separation and the thickness of the (model) polyion in its two conformations, as these two model parameters determine the polyion–mobile ion interactions under given conditions of temperature and solute concentrations. Information of this kind is of particular value in cases when the helix and/or the coil conformations are incompletely characterized.

In order to obtain the desired information on the electrostatic interactions in solutions of a ionic polymer undergoing a coil–helix transition, we have in this study measured the self-diffusion of the counterions by the Fourier transform NMR pulsed-gradient spin-echo technique.[1,2] With the advent of this rapid NMR method, the use of counterion self-diffusion as a probe of polyion–counterion interactions has become increasingly more attractive, and a number of studies concerned with synthetic polymers[2-5] as well as DNA[6] have appeared in recent years. However, to our knowledge, ours is the first study that utilizes the counterion self-diffusion method to monitor the *changes* in counterion–polyion interactions following the helix–coil transition of a polyion.

The system studied here is ι-carrageenan, a sulfated polysaccharide extracted from marine algae, which has the ability to undergo the transition from coil to helix in solution.[7] ι-Carrageenan has a repeating disaccharide structure containing one sulfate group per sugar residue (Figure 1). Under suitable conditions of polysaccharide and salt concentrations, ι-carrageenan, like the less charged κ-carrageenan (Figure 1), may form thermoreversible aqueous gels, and one particular reason for the interest in the helix conformation of the carrageenans is that the helix seems to be the "building block" required for the formation of the junctions in the physically cross-linked polymer network.[7] While the bulk of experimental results supports the notion that carrageenan, in solution as in the solid state, forms a double helix by the intermolecular association of two chains,[7-9] evidence suggesting an intramolecular helix formation in solution is continually being presented in the literature.[10-13] Inasmuch as different proposed helical structures have differing surface charge densities, they may be distinguished by the methods of our study, and we will discuss the implications of our counterion self-diffusion data in this respect below.

Experimental methods having been employed previously to obtain information on the effects of the coil–helix transition on the polyion–counterion interactions in carrageenan solutions include measurements of conductivity,[13,14] counterion activity coefficients,[14] ultrasonic absorption,[15] and counterion NMR.[12,16-19] Most of these studies[12-16] were, however, restricted to κ-carrageenan, and the conformational transition was studied only in the presence of certain ions that specifically promote the helix formation. Apart from a very recent counterion NMR relaxation study by Kvam and Grasdalen,[19] our present

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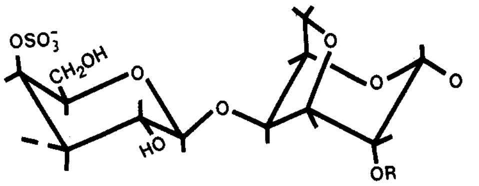

Figure 1. Repeating disaccharide structures of ι- (R = SO_3^-) and κ-carrageenan (R = H).

work seem to be the only study to date that specifically aims at directly monitoring the nonspecific electrostatic interactions of counterions with ι-carrageenan in its two solution conformations. (Only one report on the self-diffusion of counterions in carrageenan solutions has been published previously;[20] this study was, however, limited to ι-carrageenan in its coil state.) We have chosen tetramethylammonium (TMA) as the counterion in our study, since this ion has excellent NMR properties and is known[14,21] to neither interact specifically with the carrageenans nor to promote unwanted aggregation effects. Although the helix–coil transition of ι-carrageenan is not cation-specific,[22] natural ι samples always seem to contain traces of κ-carrageenan, which may aggregate in the presence of certain cations.[22,23]

In the analysis of our data, we will use theoretical results originally derived by Yoshida,[24] and later, using a slightly different approach, by Nilsson et al.[6] The theory uses the cylindrical cell model of a polyelectrolyte solution, according to which the system is divided into infinite cylindrical cells of uniform radius, each containing an infinite cylindrical model polyion of radius *a*, centered in the cell, and where the cell radius, *b*, is given by the polyion concentration. The model polyion has a smeared out surface charge density, which is given by its radius and by its length per unit charge, *l*, where the latter parameters is given by the projected charge separation of the real polyion in the conformation considered. The macroscopic counterion diffusion coefficient, *D*, in an isotropic system of such rods is obtained[6,24] as

$$D = \tfrac{1}{3}D_{\mathrm{axi}} + \tfrac{2}{3}D_{\mathrm{rad}} \qquad (1)$$

where D_{axi} and D_{rad} are the counterion diffusion coefficients, in the cell, in the directions parallel and perpendicular to the cylinder axis, respectively. An expression for the ratio D_{rad}/D_0, where D_0 is the diffusion coefficient in a dilute symmetrical electrolyte solution, is obtained from the solution of the Smoluchowski diffusion equation, under steady-state conditions, in the cylindrical cell, using the Poisson–Boltzmann approximation to obtain the concentration profile of the mobile ions. Recent comparisons[25] of the predictions of the Smoluchowski–Poisson–Boltzmann equation with the results of stochastic dynamics simulations have shown that the former equation gives an accurate description of the diffusion of monovalent counterions in inhomogeneous electrolyte systems. Very recently, the Poisson–Boltzmann cell model has also been shown[26] to give a satisfactory description of the electrostatic contribution to the difference in chemical potential between the helix and the coil conformation for carrageenans. It is therefore natural to use the same electrostatic model in the present context.

Yoshida's theoretical prediction for the counterion diffusion quotient, D/D_0, has been compared to experimental data in a few recent works,[3,5,6] and the consistent picture emerging from these studies is that the theory yields too large diffusion quotients in salt-free polyelectrolyte solutions. On the other hand, in the case of salt titrations of DNA, the agreement between theory and experimental measurements of the diffusion of monovalent cations was almost quantitative.[6] The discrepancy between theory and experiment for the salt-free case has generally been ascribed[3,6] to the assumption that D_{axi} equals D_0, which was made in all these comparisons (note that the ratio D_{axi}/D_0 is not predicted by the theory). Although this uncertainty in D_{axi} results in an uncertainty in the *level* of the diffusion quotients, previous investigations[3] still indicate that the theory is quantitatively quite successful in describing the *shift* in the diffusion quotient following a change in the polymer charge density—an observation to which we will return in the following.

II. Experimental Section

Materials. ι-Carrageenan (from *Eucheuma spinosa*, lot no. 124F-0605) was obtained from Sigma Chemical Co. ι-Carrageenan segments prepared from the same lot have been shown[22] to have a primary structure that closely approaches the ideal repeating disaccharide structure of Figure 1. Segments of enhanced structural regularity were prepared as described by Bryce et al.[8] Determinations[9,27,28] of the molecular weight of ι-carrageenan segments have yielded weight-average values in the range (35–120) × 10^3. Analyses by gel permeation chromatography in our laboratory have shown that the segmentation procedure yields samples with a reproducible but broad molecular weight distribution profile.

The carrageenan segments were dialyzed against millipore-filtered water and freeze-dried. The TMA salt form of the polyelectrolyte was obtained by ion exchange at elevated temperature. The samples used in the experiments described below were prepared by dissolving the freeze-dried TMA salt in D_2O. Sample concentrations are given as moles (dissacharide) per cubic decimeters (M), assuming ideal disaccharide molecular weights for the freeze-dried segments.

Optical Rotation. The temperature-induced conformational transition of carrageenan was monitored by optical rotation measurements at 436 nm in a Perkin-Elmer 141 polarimeter. The temperature was controlled by circulation of thermostatically regulated water through the jacketed 15-mm path length cell. Both cooling and heating runs were made, showing good reproducibility. The specific optical rotation, $[\alpha]_{436}$, is given in units of degrees/(centimeters·molar).

Self-Diffusion Measurements. Measurements of the TMA diffusion in carrageenan and reference samples were performed on a JEOL FX-100 Fourier transform NMR spectrometer as described previously.[3] Experimental results are given as diffusion quotients, D/D_0, where D and D_0 are the TMA diffusion coefficients in carrageenan and reference solutions, respectively, measured under identical conditions. As a reference, a solution of 0.11 mol dm^{-3} TMABr in D_2O was used throughout. For the purpose of evaluating D_0, this solution may be regarded as infinitely dilute.[29]

III. Experimental Results

The experimental results of this study are summarized in Figure 2, where the temperature dependences of the specific optical rotation and the TMA diffusion quotients in salt-free solutions of TMA–carrageenan at two concentrations (13 and 55 mM) are compared. (Only salt-free solutions, which give a maximum reduction in the counterion diffusion coefficients, were studied.) It is clear that the helix–coil transition of carrageenan, which is monitored by the temperature-induced shift in the optical rotation, has a marked effect on the counterion diffusion coefficient. Thus, a high content of the helical conformation, which prevails at low temperatures in the more concentrated carrageenan solution, leads to a significant lowering of the counterion diffusion quotient. On the other hand, in temperature regions when only one of the two conformations is stable, the diffusion quotient is nearly constant, which is readily understood since electrostatic interactions in aqueous solutions are approximately proportional to the absolute temperature. (The product $\epsilon_r T$, where ϵ_r is the

Table I
Calculated (See Text) and Measured Counterion Diffusion Quotients in 55 mM TMA–Carrageenan Solution

	coil[a]		single strand helix[b]		double strand helix[c]	
polyion radius given by	a, Å	D/D_0(calc)[e]	a, Å	D/D_0(calc)[e]	a, Å	D/D_0(calc)[e]
constant vol	3.46	0.825	3.73	0.796	5.28	0.628
+ contact radius	6.96	0.903	7.23	0.881	8.78	0.706
circumscribed cylinder	3.0	0.811	6.5	0.865	6.5	0.656
+ contact radius	6.5	0.896	10.0	0.915	10.0	0.732

[a] l = 5.0 Å; D/D_0(exp)[d] = 0.720. [b] l = 4.3 Å; D/D_0(exp)[d] = 0.557. [c] l = 2.15 Å; D/D_0(exp)[d] = 0.557. [d] Averages over temperature intervals 0–20 and 40–90 °C for helix and coil, respectively. [e] Calculated for 20 and 40 °C for helix and coil, respectively.

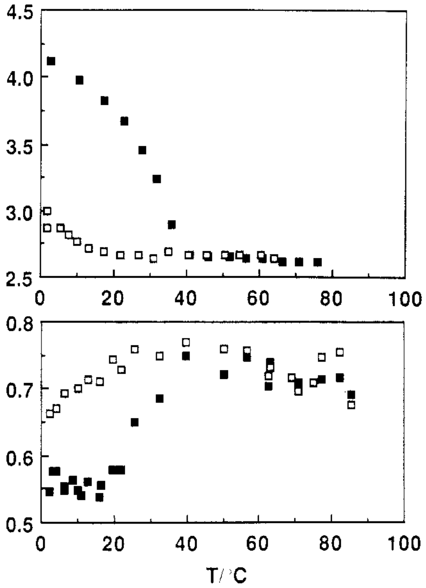

Figure 2. Specific optical rotation (upper part) and experimental counterion diffusion quotients (lower part) as functions of temperature in salt-free solutions of TMA–ι-carrageenan in D_2O. The carrageenan concentrations were 13 mM (open symbols) and 55 mM (filled symbols).

relative permittivity of water and T is the absolute temperature, is nearly constant.)

From the optical rotation data, the 13 mM carrageenan solution is seen to have a much lower helical content than the 55 mM solution at low temperatures. This feature is also reflected in a much less dramatic decrease in the TMA self-diffusion, at low temperatures, in the less concentrated sample. This well-known dependence of the helix–coil transition on polyelectrolyte concentration in salt-free samples is readily understood from electrostatic considerations.[26] From now on, we will mainly discuss the more concentrated sample, which had a helical content exceeding 90% at the lowest temperatures, as inferred from a comparison of its specific optical rotation with that obtained in the presence of 0.3 M TMABr, where the conversion to the helical state at low temperatures was complete.

IV. Model Predictions and Discussion

As mentioned in the Introduction, there is some controversy in the literature regarding the question of whether the carrageenan coil–helix transition is an intermolecular or an intramolecular process. In our opinion, all experimental observations have not yet been satisfactorily accounted for within any one proposed helical model, and therefore such models should be treated with some caution. In particular, we feel that it is important to distinguish experimental evidence bearing on the shape of the helix from evidence regarding the stoichiometry of its formation, i.e., whether the reaction is uni- or bimolecular. Thus, the experiments performed here only yield information on the *shape* of the helix conformation, as reflected in the model parameters a and l. Previously, when electrostatic models have been used in the literature to extract information on the conformation of the κ-carrageenan helix,[11-13] two possible helical shapes have been considered, one being a single helix with a conformation corresponding to one of the strands of the proposed solid-state double helix[30] and the other a "helical dimer" consisting of two such strands formed by the intermolecular association of two chains (but not necessarily equal to the double helix). Also in this work, we shall compare model predictions based on these two helical conformations, which we shall refer to as the "single strand" and "double strand" conformations, respectively. However, it should be borne in mind that, on the basis of the evidence presented here, it is impossible to distinguish the double strand conformation from, e.g., a tightly wound single helix with the same projected charge separation (sterically, such a conformation seems possible[30]) or from a double helix formed intramolecularly by the folding of the chain back on itself.

We will now analyze our data in terms of the theory described above, where the electrostatic interactions are treated within the Poisson–Boltzmann cell model. When this model is applied to a real polyelectrolyte solution, the value of the parameter l of a polyion in a given conformation may be assigned unambiguously. In the present case, the projected charge separation is obtained from fully extended molecular models and from X-ray data on oriented fibers[30] for the coil and helix models, respectively. On the other hand, the choice of a model polyion radius, a, presents a greater problem in the common cases when the thickness of the polyion is not much greater than the molecular roughness of the polyion–solvent interface. In applications where the variations in the distribution of the mobile ions following a change in charge density (e.g., in a polyacid titration experiment) of the polyion is studied, it is commonly found that the theoretical results are not so sensitive to the actual choice of the (constant) model polymer radius.[3] However, as we in the present application intend to compare two conformations with *different* radii, our choice of these radii deserves some discussion—all the more so, as we wish to compare our experimental results with theoretical predictions based on two different helical conformations.

In Table I we compare the experimentally obtained counterion diffusion quotients with those predicted, by the theoretical model specified above (further computational details are given in ref 3), for various model polyions chosen to represent the coil and the (single and double strand) helix conformations. All theoretical diffusion quotients in Table I correspond to the case $D_{\mathrm{axi}} = D_0$. Owing to the small temperature dependence of the diffusion quotients in regions outside the conformational transition, experimental data are given as averages over the temperature intervals where one conformation totally dominates over the other. For each one of the polysaccharide conformations considered, we have used two different strategies in obtaining the appropriate model polyion radius. Thus, one model radius corresponds to the (estimated) smallest cylinder circumscribing the entire molecule in each particular conformation, whereas the other is based on the requirement that the molar volume of the polyion should be independent of its conformation. In the latter case, we have used a volume, per unit charge, of the model polyion that is equal to the partial molar volume[31] of the real polysaccharide (the small difference in partial molar volume between the coil and the helix conformations, which is indicated by experiment,[32] is neglected). We also present results calculated for the cases where a contact radius of 3.5 Å of the TMA ion[33] has been added to the circumscribed cylinder or constant volume estimates of the polyion radius.

From the theoretical results of Table I, we may first conclude that the sensitivity of the calculated diffusion quotients to the value of the model polyion radius is small compared to the large effects that result from variations in the projected charge separation of the polyion. For instance, taking the coil with a = 6.5 as a reference, we see that a reduction of the radius by a factor of 2.17 (from a = 6.5 Å to a = 3.0 Å) at constant l reduces the diffusion quotient by 0.085 unit, whereas a reduction of the intercharge separation by a factor of 2.33 (from l = 5 Å to l = 2.15 Å) at constant a reduces D/D_0 by 0.24 unit. The theory thus predicts a large difference in the diffusion quotients for the single and the double strand models (with intercharge separations differing by a factor of 2), which does not disappear with any reasonable choice of radii. Conversely, no sensible choice of model radii will yield a theoretical diffusion quotient for the single strand which differs greatly from that of the coil, owing to the small difference in projected charge separation of the two conformations.

When the theoretical results for the coil and the single strand are compared, it is, furthermore, seen that the circumscribed cylinder estimate of the single strand radius actually yields a counterion diffusion quotient that is *larger* than that of the model coil system. This is due to the fact that, when the charges of the single strand are smeared out over a cylinder that is large enough to contain the entire structure, the surface charge obtained is actually lower than that of the corresponding model coil. This result, however, indicates that the circumscribed cylinder method to estimate the model radius of a noncompact polyion conformation may yield artificial results. (That the result is indeed an artifact may be realized from the following considerations. The single strand is a fully realizable element in the configurational space available to carrageenan in solution. If we were to evaluate its electrostatic free energy[26] with its dimensions given by the circumscribed cylinder method, we should find that the single strand conformation would be *favored*, electrostatically, relative to the random coil, although the polyion charges are actually brought closer together in the single strand conformation.) The constant volume method, on the other hand, will always yield surface charge densities (and electrostatic free energies) that vary monotonically with the projected charge separation of the polyion conformation. (Whether this method also leads to quantitatively correct estimates of the electrostatics of noncompact polyion conformations is, of course, a different question.)

When the theoretically predicted diffusion quotients are compared with those obtained by experiment (Table I), we can immediately conclude that the single strand may be ruled out as a possible helix conformation under the conditions of our study. This follows since the difference in the counterion diffusion quotients obtained experimentally for the helix and the coil solutions may not be accounted for theoretically on the basis of a single strand. On the other hand, this *difference* is reproduced almost quantitatively by the theoretical differences between coil and double strand diffusion quotients, although the *absolute* diffusion quotients given by theory are too high for both conformations. (This conclusion holds regardless of whether a contact radius for the counterion is included in the effective polyion radius or not, as long as the radii of both conformations are obtained in the same way.) This discrepancy between theory and experiment is, however, consistent with the results from a previous investigation of polyacid solutions,[3] where experimental and theoretical counterion diffusion quotients, obtained in the same way as those reported here, were compared. In the latter work, it was found that the theoretically predicted diffusion quotients were always too large, compared with experiment; nevertheless, the *changes* in diffusion quotients resulting from changes in polyion charge (following neutralization of the polyacids) were reproduced rather accurately.

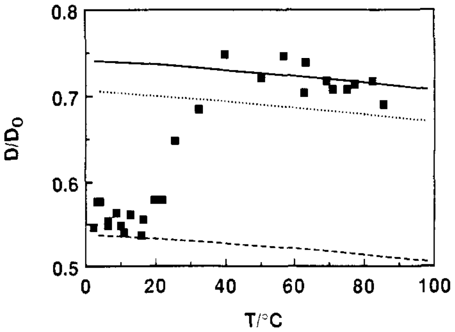

Figure 3. Experimental (squares) and calculated counterion diffusion quotients in 55 mM ι-carrageenan as functions of temperature. The calculated curves represent model predictions for the coil (solid line) and the single (dotted line) and double strand (dashed line) helices. Model calculations are based on polyion radii obtained from the partial molar volume of ι-carrageenan (see Table I). A constant value of 0.095 has been subtracted from all calculated data (see text).

For the system studied here, a similarly good agreement between experiment and theoretical predictions for the case of a coil-to-double strand transition may be seen in Figure 3, where we have simply subtracted a constant (0.095 unit) from all D/D_0 values calculated with polyion radii obtained by the constant volume method without an added contact radius. (Almost identical results are obtained if 0.173 unit is substrated from the D/D_0 values obtained with a contact radius of 3.5 Å; cf. Table I). At least in part, this difference between theory and experiment may be understood as resulting from the assumption of unrestricted axial counterion diffusion ($D_{\text{axi}} = D_0$; cf. section I), which was made in the calculations. In a real system, this component of the diffusivity should also be reduced as a result of the accumulation of counterions close to the polyion—regardless of whether the dominant contribution to this effect may be ascribed to the high concentration[29] as such or to dynamic correlations[25] of the mobile ions with the polyion. In the absence of a theory that makes quantitative predictions of D_{axi} and its dependence on the polyion charge density, we may regard the subtraction of a constant number from the theoretical diffusion quotients, made in Figure 3, as an ad hoc amendment to the Yoshida theory, which finds some support in the results of previous comparisons between experiment and theory.[3]

So far, we have not presented any theoretical results for the more dilute system of Figure 2. Calculations for this system gave D/D_0 values lower than those of the more concentrated system by a roughly constant amount of 0.06 unit for all the different conformations. This result is not supported by the experimental results (Figure 2) where no significant concentration dependence is to be seen at high temperatures, when both solutions contain coils only. However, again we can compare this result with the results obtained in previous systematic studies[3,4] of synthetic polyions. Thus, in Figure 3 of ref 3, it is seen that a decrease in D/D_0 on dilution, in accordance with Yoshida's theory, occurs only at high concentrations; at very low concentrations, D/D_0 instead *increases* rapidly on dilution. Between these regimes of opposite concentration dependences, there is a region (around 0.03 M for the particular polymethacrylate sample of ref 3) where the concentration dependence is almost flat. The position as well as the extent of this region is dependent on the molecular weight of the polyion, as may be seen in the results of ref 4, where the concentration dependence of the TMA diffusion in polyacrylate samples of different molecular weights was studied. Thus, for a polymethacrylate sample with a molecular weight of 5000, no significant variation in D/D_0 was seen in the concentration range 0.01–0.1 M (Figure 3 of ref 4). These deviations, at low and intermediate polyelectrolyte concentrations, of the experimental results from the theoretical predictions may in all probability be attributed to end effects for polyions of finite length[4,5] (which become increasingly important on dilution), as the theoretical calculations are based on infinitely long polyions.

V. Conclusions

We find that measurements of the counterion self-diffusion may yield valuable information on the helix–coil transition of ionic polymers, making possible the distinction between different a priori possible helix conformations. The method, which has not been applied to similar studies previously, is expected to give useful information also in other polyelectrolyte systems where a coil–helix transition occurs, in solution, under conditions when the amount of added salt is low enough so that the influence of the polyion on the counterion diffusion is substantial. Compared with conductivity measurements, which may yield similar information,[13,14] the method used here has the advantage of allowing selective studies of particular ionic species in mixed counterion systems.

In the case studied here, experimental data on the self-diffusion of counterions in ι-carrageenan, analyzed within the Poisson–Boltzmann cell model, give results that are entirely consistent with a transition of ι-carrageenan in solution from a random coil (at high temperatures) to a helix (at low temperatures), which has the same dimensions as the proposed solid-state double helix.[30] This conclusion is further corroborated when the systematic quantitative differences between theory and experiment found in this study are compared with similar discrepancies found in a previous study on synthetic polyacids.[3] On the other hand, under the conditions of this study, a helix conformation corresponding to only one of the strands of the proposed solid-state double helix may be ruled out.

The conclusions regarding the shape of the ι-carrageenan helix presented here are in good agreement with the conclusions drawn by Kvam and Grasdalen[19] from their studies of the paramagnetically induced NMR relaxation of TMA in solutions of ι-carrageenan with mixed counterions (TMA and Mn^{2+}). Similarly, a thermodynamic analysis[26] of the dependence of the ι-carrageenan coil–helix transition on carrageenan concentration and counterion valency, based on the same electrostatic model as was used here and in ref 19, gives support to the same helical shape. At least under the rather wide range of conditions spanned by these three studies, a consistent picture of the shape of the ι-carrageenan helix therefore applies.

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