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Helix–Coil Transitions of Ionic Polysaccharides Analyzed within the Poisson–Boltzmann Cell Model. 2. Effects of Salt Concentration on the Thermal Transition

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ABSTRACT: The temperature-induced helix–coil transitions of κ- and ι-carrageenan (two charged polysaccharides) in the presence of varying concentrations of salts (1:1 and 2:1 electrolytes) have been studied. Apart from the well-known effect of salt on the transition temperature, the polyion–polyion interactions are found to influence the width of the conformational transition at low salt concentration, which is of relevance for the interpretation of the cooperativity of the transition from such experimental data. In the theoretical analysis, based on the full Poisson–Boltzmann equation in a cylindrical cell model, electrostatic polyion–polyion interactions are shown to affect the propagation step of the transition. With the assumptions that the helix initiation step as well as the nonelectrostatic part of the transition entropy and enthalpy (the latter used as a fitting parameter) all are independent of temperature or ionic content, the model predictions of effects of 1:1 electrolyte on the transition of ι-carrageenan were in quantitative agreement with experiment. The use of the same value of the transition enthalpy in the case of added 2:1 electrolyte yielded a semiquantitative agreement with experiment.

I. Introduction

Natural polymers that undergo helix–coil transitions in solution are typically polyelectrolytes (e.g., DNA, most polypeptides, and many polysaccharides), and an evaluation of the effects of electrostatic interactions on such transitions is therefore an important problem, which, nevertheless, has remained largely unsolved. Remarkably few attempts have been made to apply the full Poisson–Boltzmann equation to helix–coil transitions of polyelectrolytes;[1-3] previous attempts have largely been limited to various approximative expressions such as the linearized Poisson–Boltzmann equation[4] or Manning's limiting laws.[5] In a series of papers we attempt to improve on this situation, analyzing the electrostatic effects on helix–coil transitions of certain well-characterized ionic polysaccharides within the so-called Poisson–Boltzmann cell model[6-8] (PBCM; this model was also used in a very recent work by Jamnik and Bratko[2]). The approach is here to calculate the electrostatic contribution to the chemical potential of the polyion from the full Poisson–Boltzmann equation in a cylindrical cell model, with the polymer chain approximated as an infinitely long rod with uniform surface charge density, taking the finite polymer concentration into account and thus treating the polyion–salt interactions and the polyion–polyion interactions in a self-consistent way.

Our previous paper[1] was devoted to the analysis of the effects of counterion valency and polyelectrolyte concentration on the transition, and it was found that the PBCM could give a qualitatively correct and semiquantitatively accurate description of experimental observations, including some anomalous effects occurring in systems containing counterions of mixed valency that could not be accounted for by the more approximate theories. In the present paper, we will focus on the effects of added salt on the temperature-induced transition; we will thus analyze the *shift* along the temperature axis as well as the change in *sharpness* of the transition which results from changes in electrolyte content. While the former effect of salt concentration is at least qualitatively well-understood, the latter effect has not been satisfactorily accounted for previously, except for its brief mention in our previous work.[1] Such salt-dependent effects on the sharpness of the transition have been observed before but were then taken to be due either to an ionic-strength dependence in the cooperativity[9] or, in the case of divalent counterions, to ion condensation[10] in Manning's polyelectrolyte theory.[5]

As in our previous investigation, we have here chosen to study κ- and ι-carrageenan[11] (Figure 1), two charged sulfated polysaccharides with one and two charges per repeating (disaccharide) unit, respectively. Apart from being good model substances shedding light on general aspects of helix–coil transitions of polyelectrolytes,[1] the carrageenans are also interesting in their own right, being well-known gel-forming (under suitable conditions) polysaccharides where the helix formation is believed to be the first step in the formation of a gel network. Furthermore, the salt effects on the helix–coil transition of κ-carrageenan in particular have been extensively studied previously and analyzed within the framework of electrostatic theory (especially Manning's limiting laws);[12-17] yet there are considerable discrepancies in the interpretations proposed, especially regarding the multiplicity of the helical conformation (a single or a double helix). It should therefore be illuminating to use other theoretical approaches to this particular system and to see to what extent quantitative predictions are model dependent.

II. Theoretical Model

1. General Considerations. The theoretical model, together with its underlying assumptions, has been described[1] in detail previously and will therefore be reviewed only briefly here. The helix–coil transition is analyzed in terms of the conventional Zimm–Bragg model,[19,20] according to which it is assumed that each repeating unit of the polymer chain can be either in the helix or in the coil conformation with an entropy of mixing between helix and coil units. Two parameters are needed to describe the transition, one initiation parameter, σ, and one propagation parameter, s, with $\ln \sigma s$ and $\ln s$ corresponding to the free energies of forming the first and subsequent helical units, respectively. In the case of polyions, the propagation parameter may be divided into one electrostatic and one nonelectrostatic part:

$$kT \ln s = m(\Delta\mu_{\mathrm{el}} + \Delta\mu_{\mathrm{nonel}}) \tag{1}$$

where m is the number of charged groups per repeating unit and $\Delta\mu_{\mathrm{el}}$ and $\Delta\mu_{\mathrm{nonel}}$ are the electrostatic and the nonelectrostatic contributions to the free energy per unit polyion charge. (In the case of double-helix formation it is customary to define the propagation parameter as corresponding to the free energy of forming one *pair* of repeating units in the helix conformation; however, in this

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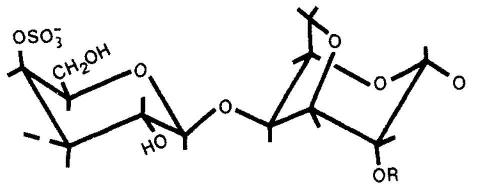

Figure 1. Repeating disaccharide structures of κ- (R = H) and ι- (R = SO_3^-) carrageenan.

paper all interactions are given per unit polyion charge.) Here it is assumed that the nonelectrostatic part, $\Delta\mu_{\rm nonel}$, is independent of the presence of salt; it will, however, be a function of the temperature. This temperature dependence is taken care of by simply dividing $\Delta\mu_{\rm nonel}$ into one enthalpic and one entropic part, each of which is taken to be temperature independent:

$$\Delta\mu_{\rm nonel} = \Delta H_{\rm nonel} - T\Delta S_{\rm nonel} \tag{2}$$

This temperature dependence in $\Delta\mu_{\rm nonel}$ represents our only present extension of the model described previously.[1]

The cooperativity of the transition is governed by the initiation parameter, σ, which is, as before, taken to be independent of electrolyte concentration and temperature.[1,19,20] The value of the initiation parameter can be related to the width of the conformational transition as a function of, e.g., the temperature. However, it is important to realize that also factors other than the cooperativity influence the width of the conformational transition as will be discussed in the following sections.

2. Electrostatic Model. In the PBCM, the electrostatic free energy of each conformation is calculated from the Poisson–Boltzmann equation in a cylindrical cell model.[8,21] Here the solution is divided into uniform cylindrical cells, containing water and mobile ions, with the polymer chain centered in the cell and approximated as a cylindrical rod of radius a having a uniform surface charge density σ. The radius of the cell, b, is given by the polymer concentration and, for systems containing both coils and helices, by eq 11–13 below. In cylindrical symmetry the Poisson–Boltzmann equation becomes

$$\frac{1}{r}\frac{\mathrm{d}}{\mathrm{d}r}\left(r\frac{\mathrm{d}\phi}{\mathrm{d}r}\right) = -\frac{e}{\epsilon_r\epsilon_0}\sum c_{i0}z_i \exp(-e\phi z_i/kT) \qquad a < r \le b \tag{3}$$

to be solved with the boundary conditions $\mathrm{d}\phi/\mathrm{d}r(b) = 0$ and $\mathrm{d}\phi/\mathrm{d}r(a) = -\sigma/\epsilon_0\epsilon_r$ ($\phi(b)$ is taken to be zero by convention). Here c_{i0} is the concentration of mobile ions of charge z_i at $r = b$ (the other symbols carry their usual meaning; a list of symbols is given in ref 1). Given the electrostatic potential $\phi(r)$ the electrostatic free energy can be calculated as[21]

$$G_{\rm el} = E_{\rm el} - TS_{\rm mix} \tag{4}$$

with

$$E_{\rm el} = (\epsilon_r\epsilon_0/2)\int(\nabla\phi)^2\,\mathrm{d}V \tag{5}$$

$$TS_{\rm mix} = -N_{\rm A}kT\sum_i\int c_i[\ln\,(c_i/c_{\rm solvent}) - 1]\,\mathrm{d}V \tag{6}$$

Here $E_{\rm el}$ is the electrostatic interaction energy and $S_{\rm mix}$ the entropy of mixing due to the distribution of mobile ions in the cell. The chemical potentials are obtained by differentiating the free energy, leading to[22]

$$\mu_{\rm solvent} = -kT\sum c_{i0}/c_{\rm solvent} \tag{7}$$

$$\mu_i = kT\,\ln\,(c_{i0}/c_{\rm solvent}) \tag{8}$$

(single ion potential of mobile ionic species i) and

$$\mu_{{\rm el,p}i} = -e\phi(a) - E_{\rm el} + kTV_{\rm solvent}N_{\rm A}\sum(c_{i,\rm av} - c_{i0}) \tag{9}$$

Table I
Carrageenan Model Parameters (See Text)

	coil		helix	
	l, Å	*a*, Å	*l*, Å	*a*, Å
κ-carrageenan	10	3.3	4.1	5.1
ι-carrageenan	5	3.5	2.2	5.3

Here $\mu_{{\rm el,p}i}$ is the electrostatic chemical potential of the polymer per unit charge, $V_{\rm solvent}$ is the volume of solvent in the cell per polymer charge, and $c_{i,\rm av}$ is the average concentration of mobile ionic species i in the cell. The electrostatic chemical potential difference between the two conformations is then given by[1]

$$\Delta\mu_{\rm el} = \mu_{{\rm el,p}i}({\rm coil}) - \mu_{{\rm el,p}i}({\rm helix}) \tag{10}$$

The above expression is valid if the helix cells and the coil cells are at equilibrium with each other, i.e., if the chemical potentials of the mobile components are the same for both cells or, equivalently

$$c_{i0}({\rm helix}) = c_{i0}({\rm coil}) \tag{11}$$

For a complete specification of the system, two additional conditions are needed to give the correct overall amounts of salt and solvent in the system, i.e.

$$c_{i,\rm av}({\rm coil})(1-p) + c_{i,\rm av}({\rm helix})p = \langle c_i\rangle \tag{12}$$

$$V_{\rm solvent}({\rm coil})(1-p) + V_{\rm solvent}({\rm helix})p = \langle V_{\rm solvent}\rangle \tag{13}$$

Here p represents the fraction of units in the helix conformation, $c_{i,\rm av}$ is the cell-averaged concentration of the mobile ionic species i, and the brackets designate global averaging.

3. Model Parameters. The dimensions of either conformation are given by the length per unit charge, l, and the radius, a. The length per unit charge is taken from X-ray diffraction data[23,24] on oriented fibers for the helix conformation and from molecular models for the coil conformation. In the calculation of the rod radius, we have taken the volume per unit charge of the model polyion to be independent of its conformation and equal to the corresponding experimentally determined partial molar volume.[25,26] The model parameters (which are identical with those used in our previous studies[1,29]) are listed in Table I. The choice of parameters for the ordered conformation is somewhat controversial; although a large number of experimental studies[11,18,27-29] support the notion that the conformation in solution is the same as that of the proposed solid-state double helix,[23,24] a single helical structure (in solution) consisting of one strand of the solid-state double helix (and therefore with half the linear charge density) has also been proposed.[12,15,16] Our detailed arguments for using model parameters based on the double helical conformation are given in section V.

III. Experimental Section

1. Materials. ι-Carrageenan samples (obtained from Sigma Chemical Co, lot no. 124F-0605) were segmented as described by Bryce et al.[18] The carrageenan segments were dialyzed against millipore-filtered water, ion-exchanged at elevated temperature, and freeze-dried as described previously.[1] Sample concentrations are given as moles (disaccharide) per dm^3 (M) assuming ideal disaccharide molecular weight for the freeze-dried segments.

2. Methods. The helix–coil transition of carrageenan was monitored[11] by optical rotation (OR) measurements at 436 nm on a Jasco DIP 360 polarimeter. The temperature was controlled by circulating thermostatically regulated water through the

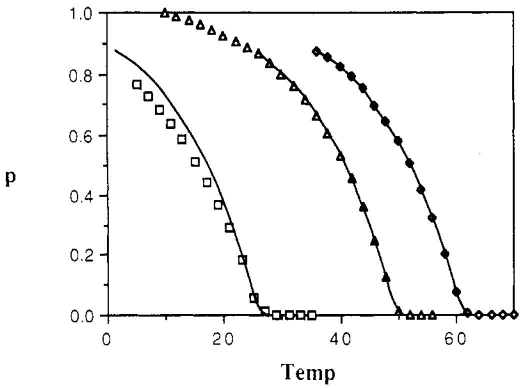

Figure 2. Thermally induced helix–coil transition at different concentrations of NaCl. The solid lines have been obtained by drawing a line through the experimental points at 0.2 M NaCl, which has then been parallel transferred to match the onset of conformational ordering at lower salt concentrations to illustrate the change in sharpness for the transition. Experimental conditions: 49 mM Na-ι-carrageenan; salt concentrations: salt free (□), 0.1 M NaCl (△), 0.2 M NaCl (◇).

jacketed cell. The evaluation of the helix content by OR was made by using

$$p = (\alpha - \alpha_{\text{coil}})/(\alpha_{\text{helix}} - \alpha_{\text{coil}}) \tag{14}$$

$$\alpha_{\text{helix}} = c_p d[\alpha]_{\text{helix}} \tag{15}$$

$$\alpha_{\text{coil}} = c_p d[\alpha]_{\text{coil}} \tag{16}$$

where p is the fraction of helix conformation, α the measured optical rotation at path length d, c_p the polymer concentration, and $[\alpha]$ the specific optical rotation. Here $[\alpha]_{\text{coil}}$ was determined from the plateau value in the optical rotation at high temperatures, whereas $[\alpha]_{\text{helix}}$ was estimated from the highest observed α value (the curve with 0.1 M NaCl in Figure 3). This estimated value of $[\alpha]_{\text{helix}}$ may actually be slightly too low (the experimentally obtained curves have not leveled out completely); however, this will only marginally affect our results.

IV. Results and Model Interpretations

In Figure 2 the effects of added NaCl on the thermally induced helix–coil transition of ι-carrageenan are depicted (for ι-carrageenan the transition is completely reversible), displaying the familiar features of salt effects on polyelectrolyte helix–coil transitions: The transition is shifted toward higher temperatures with increasing salt content (this is a general feature only for systems with one type of counterion[1]), and the transition is also seen to become sharper as the electrolyte concentration is increased. The latter effect is small for ι-carrageenan, which may be the reason it seems not to have been noted previously; nevertheless, it is clearly significant. It is our objective to interpret both these effects, using the model presented above, in the following.

1. Salt-Induced Temperature Shift of the Conformational Transition. In the theoretical analysis of the temperature dependence it is first necessary to evaluate the nonelectrostatic transition enthalpy. This can be extracted from the salt concentration induced temperature shift in the coil–helix transition, as obtained from experiment, via our model, which predicts the salt concentration dependence of $\Delta\mu_{\text{el}}$. Here we will make use of the fact that as long as the helix initiation is independent of salt concentration and temperature (which is a basic assumption of our model), any given helical content of the system (regardless of the conditions under which it is obtained) corresponds to a unique value of the propagation parameter, s. (In the case of a double helix, the polymer concentration must also be constant.) Hence, when comparing transition curves obtained at different salt concentrations, the following relation holds for points of the different curves corresponding to the *same helical content* (cf. eq 1 and 2):

$$(\ln s)_{p=\text{const}} = [m(\Delta\mu_{\text{el}} + \Delta H_{\text{nonel}} - T\Delta S_{\text{nonel}})/kT]_{p=\text{const}} = \text{constant} \tag{17}$$

or

$$(\Delta\mu_{\text{el}}/kT)_{p=\text{const}} + (\Delta H_{\text{nonel}}/kT)_{p=\text{const}} = \text{constant} \tag{18}$$

where the last step follows from the assumption that ΔS_{nonel} is constant. Thus, a plot of $\Delta\mu_{\text{el}}/kT$, calculated for different experimental conditions all yielding the same helical content, versus the inverse of the corresponding experimental temperature should result in a straight line with the slope $-\Delta H_{\text{nonel}}/k$. (The advantage of this analysis as compared to earlier treatments[2,3,12,18,30] is that it eliminates the need for unnecessary approximations, such as postulating the conformational transition to be a two-step all-or-none transition or that the free energy difference equals zero (i.e., $s = 1$) at the transition midpoint.) Note that relation 18 holds for any (arbitrary) helical content. As the point of onset of helix formation experimentally is quite well defined (cf. Figure 2), we generally prefer to use this point in our analysis. The result of such an analysis for ι-carrageenan with monovalent counterions is displayed in Figure 3, verifying the linear dependence of eq 18 and yielding a value of 8.6 kJ/mol (per mole of polyelectrolyte charges) for ΔH_{nonel}.

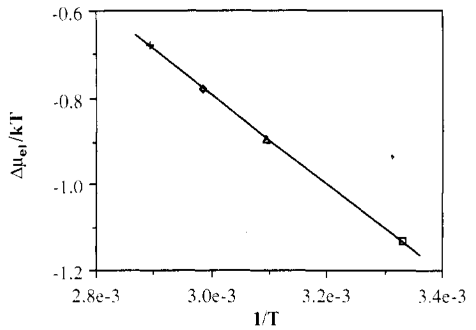

Figure 3. $\Delta\mu_{\text{el}}/kT$ calculated for the onset of conformational ordering versus the inverse of the corresponding (experimental) temperature. Experimental conditions: 49 mM Na–ι-carrageenan, salt concentrations: salt free (□), 0.1 M NaCl (△), 0.2 M NaCl (◇), 0.4 M NaCl (+).

Table II
Nonelectrostatic Transition Enthalpies of Carrageenans Evaluated from Experimental Data by Using Polyelectrolyte Theory (See Text).

polysaccharide	added electrolyte	transition enthalpy, kJ/mol PBCM	transition enthalpy, kJ/mol Manning
κ-carrageenan	1:1	12.7	23.8
	2:1	7.0	6.9
ι-carrageenan	1:1	8.6	10.9
	2:1	6.8	8.9

The same kind of analysis was performed for ι-carrageenan in the presence of 2:1 electrolyte (CaC$_2$) and, also, for κ-carrageenan in the presence of 1:1 and 2:1 electrolytes. The results are summarized in Table II. (The corresponding plots are not shown since they all have the same appearance as Figure 3. Experimental data for κ-carrageenan were taken from the work of Rochas and Rinaudo (Figure 7 in ref 31, curves for Na$^+$ and divalent counterions), and in the corresponding calculations we used infinitely dilute systems with ionic activities equalling the "total ionic concentrations" (C_T) as given in ref 31.) For comparison, the transition enthalpies have also been calculated by Manning's limiting laws.[5] According to Manning's treatment, $\Delta\mu_{el}$ is given by[5]

$$\Delta\mu_{el}/kT = \text{const}(z) + (1/(2z^2))(1/\xi_{coil} - 1/\xi_{helix}) \ln c_{salt} \tag{19}$$

This expression was derived for conditions when the linear charge densities, ξ_{coil} and ξ_{helix}, exceed the critical value $1/z$ (where z is the counterion valency) and when only one kind of counterion is present, at a concentration largely exceeding that of the polyion. The constant depends on the counterion valency but not on the salt concentration, and its value is therefore of no consequence for the determination of ΔH_{nonel}. The above conditions regarding the linear charge densities are satisfied in all cases except for κ-carrageenan with monovalent counterions where $\xi_{coil} < 1$ and $\xi_{helix} > 1$; in the latter case $\Delta\mu_{el}$ becomes[5,12]

$$\Delta\mu_{el}/kT = \text{const} + \tfrac{1}{2}(2 - \xi_{coil} - 1/\xi_{helix}) \ln c_{salt} \tag{20}$$

The ΔH_{nonel} values obtained from Manning's limiting laws (Table II) differ from the values predicted by the PBCM; however, the differences are small except for the case of κ-carrageenan with monovalent counterions. When discussing Table II, it should also be recalled that a basic assumption of both models is that the nonelectrostatic transition enthalpy is independent of the electrolyte content of the system. Thus, ideally ΔH_{nonel} (1:1) and ΔH_{nonel} (2:1) should be the same for a given polysaccharide.

Ideally, ΔH_{nonel} can be compared with the calorimetric transition enthalpy through the relation

$$\Delta H_{cal} = \Delta H_{el} + \Delta H_{nonel} \tag{21}$$

where the electrostatic enthalpy is given by[32]

$$\Delta H_{el} = \Delta E_{el}(1 + (T/\epsilon_r)\ \partial\epsilon_r/\partial T) \approx -0.36\Delta E_{el} \quad \text{(at 25 °C in H}_2\text{O)} \tag{22}$$

Since ΔE_{el} is typically of the order of 0.3 kJ/mol, the electrostatic transition enthalpy is negligible compared to ΔH_{nonel}. Consequently

$$\Delta H_{cal} \approx \Delta H_{nonel} \tag{23}$$

Unfortunately, the values of ΔH_{cal} found in the literature vary considerably, ranging between 2 and 8 kJ/mol and 3 and 24 kJ/mol for ι-[28,30,33,38] and κ-carrageenan,[13-17,28,34,35,38] respectively. For κ-carrageenan this variation may in part reflect anion-specific effects[35] which have not been taken into account in the above treatment; however, different values have also been reported by different authors for systems of closely similar composition. Also, the κ-carrageenan transition is markedly sensitive toward the presence of certain cations (K^+,Cs^+),[31] with other cations (Li^+,Na^+,$(CH_3)_4N^+$) having almost identical effects on the conformational transition,[31] indicating that the latter ions interact only through nonspecific Coulomb interactions.

Once the transition enthalpy has been established, it is, in principle, possible to calculate the entire temperature dependence of the helix–coil transition in our model. Specifically, we have in the following chosen to analyze the transition of ι-carrageenan in this way, to avoid the problem of ion specificity (cf. above; marked ion specific effects, analogous to those observed for κ-carrageenan, are absent in the case of ι-carrageenan[27,36]) and also intermolecular aggregation which occurs and may influence the transition curves in the case of κ-carrageenan. The transition enthalpy for ι-carrageenan is taken to be 8.6 kJ/mol as obtained from the data in Figure 3. (This value will vary somewhat with the model parameters given in Table I but not critically so. For instance, if the helix radius is increased by 1 Å, the enthalpy decreases with 1 kJ/mol.)

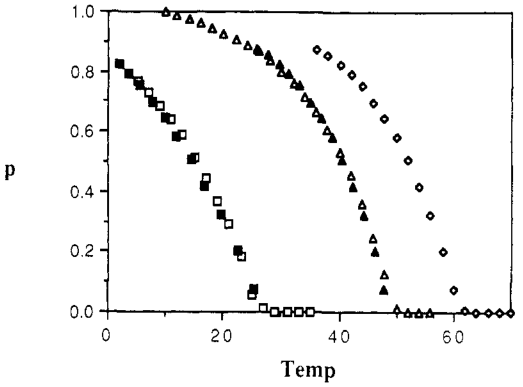

Figure 4. Conformational transition as a function of temperature at different salt concentrations for 49 mM Na–ι-carrageenan. Open and filled symbols indicate experimental and calculated points, respectively. Salt concentrations: salt free (□), 0.1 M NaCl (△), 0.2 M NaCl (◇).

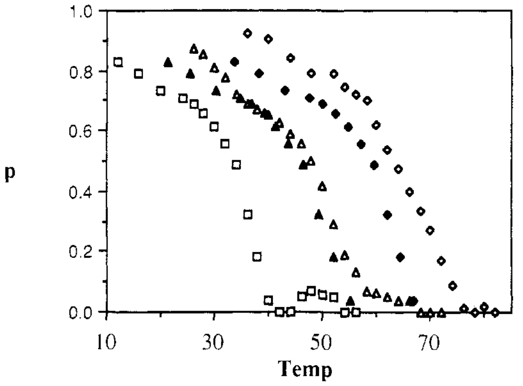

Figure 5. As in Figure 4 but with 3.9 mM Ca–ι-carrageenan. Salt concentrations: 0.1 M NaCl (□) (no Ca^{2+} was present), salt free (△), 3 mM $CaCl_2$ (◇).

For natural samples, like our samples of ι-carrageenan, one extra complication arises because of the polydispersity, making it difficult to calculate the shape of the transition curve and also to evaluate the initiation parameter. ι-Carrageenans of enhanced structural regularity, obtained through the segmentation procedure used here,[18] are known to be quite polydisperse.[37]) It is, however, possible to take one arbitrary curve as a reference curve and then calculate how this curve is shifted in temperature as the salt concentration is varied. With the condition in eq 18, a new temperature can be calculated for each point in the reference curve, subject to the conditions of constant degree of helix conformation and polymer concentration. With monovalent counterions, using the transition enthalpy obtained above, this procedure gives quantitative agreement with experimental data as is evident from Figure 4. (The curve with 0.2 M NaCl was used as a reference curve.)

In a similar experiment with divalent counterions it was necessary to use a much lower polymer concentration to obtain the entire transition below 100 °C, and owing to the low concentration there is some noise in the experimental data presented in Figure 5. Since there is a trivial polymer concentration dependence in the conformational transition (as has been observed earlier for κ- and ι-carrageenan[18]) consistent with the proposed double helix as the ordered structure,[11,18,28,29] it was necessary to use a reference curve obtained at the same concentration. (In our previous paper in this series[1] no concentration dependence was found in the presence of excess salt; this result was however obtained in a much more narrow range of concentrations

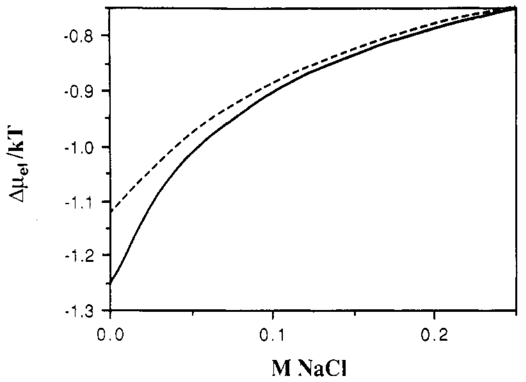

Figure 6. Calculated values of $\Delta\mu_{el}/kT$ versus salt concentration for 49 mM Na–ι-carrageenan. The solid and the dotted lines represent 100% helix conformation and 100% coil conformation, respectively.

(20–80 mM of repeating units).) The curve with Na–ι-carrageenan in 0.1 M of NaCl was therefore taken as a reference curve, and the calculated points (still using $\Delta H_{nonel} = 8.6$ kJ/mol) are seen to predict a somewhat lower transition temperature for samples with divalent counterions than is actually observed (Figure 5). This is consistent with the difference in ΔH_{nonel} in Table II for 1:1 and 2:1 electrolytes and it is also in agreement with the results in our previous paper,[1] where it was found that the theoretically predicted concentration of divalent ions required to induce the transition at a given temperature was slightly too large. However the very effective helix-stabilizing ability of divalent ions is correctly reproduced without invoking any special mechanism.

2. Electrostatic Effects on the Sharpness of the Transition. Another interesting effect of adding salt (apart from the shift in temperature) is that the transition sharpens at higher ionic strength when polyion–polyion interactions are reduced (cf. Figure 2). As pointed out above, this phenomenon has been observed before for DNA but was there attributed to either an ionic-strength dependence in the cooperativity[9] in the transition or (in the case of divalent counterions) to ion condensation[10] in terms of Manning's polyelectrolyte theory. From the results of Figure 4 it is evident that the PBCM predicts this effect with no special assumptions, and we will now discuss this general feature of helix–coil transitions of polyelectrolytes in some detail.

The conditions in eq 11–13 take care of the electrostatic polyion–polyion interactions consistently within the PBCM. The effect of these interactions will be to introduce a polyelectrolyte concentration dependence in $\Delta\mu_{el}$, as has been analyzed previously,[1] and, in addition, a dependence on the helical content in the system. The simplest way of understanding the helical content dependence in $\Delta\mu_{el}$ is to consider a salt-free system. The counterion activity is first calculated for a system with 100% coil conformation and 0% helix conformation. Through the condition in eq 11, this counterion activity also determines the electrostatic free energy and the cell radius of the first helix formed, at infinite dilution, in this all-coil system. If a system with 100% helix conformation (of the same polymer concentration) is then analyzed analogously, it turns out that the radius of the helical cell will change somewhat, but more important is that the counterion activity will decrease due to the stronger electrostatic attraction between the helix and its counterions. This decrease in counterion activity on increasing the helix content is unfavorable for both conformations, but since the helix has a higher charge density than the coil conformation, the electrostatic free energy varies more rapidly for the helix as the counterion activity decreases. The net effect is that the helical conformation is electrostatically disfavored. The resulting dependence on helical content in $\Delta\mu_{el}$ is displayed in Figures 6 and 7 under conditions corresponding to the experimental data in Figure 4. At high salt concentrations the dependence on helical content will disappear since the polyelectrolyte does not contribute significantly to the ionic activities anymore. In this limit the electrostatic part of the polymer concentration dependence also disappears for the same reason.

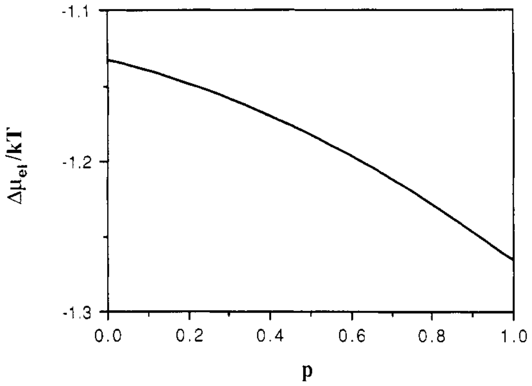

Figure 7. Calculated values of $\Delta\mu_{el}/kT$ versus helix content for 49 mM Na–ι-carrageenan (salt free).

Formally, the effect of electrostatic polyion–polyion interactions on the thermal transition can be analyzed as follows. The helical content p can generally be written as a function of the helix initiation and propagation parameters, i.e.

$$p = g(\ln s,\sigma) = g[\ln s(p,T),\sigma] \qquad (24)$$

where we have made use of the fact that s may be written as a function of p and T. In the case of single helix formation σ is here (as usual) taken to be constant; for double-helix formation, however, it will be a function of the polymer concentration as noted above. Differentiation of the last member of eq 24 with respect to T yields

$$\partial p/\partial T = (\partial g/\partial \ln s)(\partial \ln s/\partial T)\{1/[1 - (\partial g/\partial \ln s)(\partial \ln s/\partial p)]\} \qquad (25)$$

Looking at each derivative in turn, $\partial g/\partial \ln s$ is a complicated function (related to the cooperativity) except in some special cases such as at the transition midpoint ($p = {}^1/_2$) for single-helix formation in the limit of infinite molecular weight. In the latter case, the derivative becomes[20]

$$\partial g/\partial \ln s = 1/(4\sigma^{1/2}) \qquad (26)$$

The other derivatives are

$$\partial \ln s/\partial T = m[\partial(\Delta\mu_{el}/kT)/\partial T + \partial(\Delta\mu_{nonel}/kT)/\partial T] = -m[(\Delta H_{el}/kT^2) + (\Delta H_{nonel}/kT^2)] \approx -m(\Delta H_{nonel}/kT^2) \qquad (27)$$

(ΔH_{el} is negligible), and

$$\partial \ln s/\partial p = m[\partial(\Delta\mu_{el}/kT)/\partial p] \qquad (28)$$

The derivative $\partial \ln s/\partial p$ has its physical origin in the long-range electrostatic polyion–polyion interactions, and it has to be evaluated numerically from the cylindrical PBCM. Since the strength of electrostatic interactions increase on helix formation due to the increase in charge density, the derivative $\partial \ln s/\partial p$ is always negative. Therefore (since $\partial g/\partial \ln s$ is always a positive quantity), the effect of $\partial \ln s/\partial p$ will be to reduce the value of $\partial p/\partial T$ (i.e., the transition broadens), according to eq 25. However,

Table III
Helix-Coil Transition Broadening Factor (η, See Text) Calculated at the Transition Midpoint Temperature (T_m)

49 mM Na-ι-Carrageenan

T_m, °C	[NaCl], M	η
15	0	0.61
41	0.1	0.92
52	0.2	0.96

3.2 mM Ca-ι-Carrageenan

T_m, °C	[CaCl$_2$], mM	η
48	0	0.81
63	3	0.98

3.2 mM Na-ι-Carrageenan

T_m, °C	[NaCl], M	η
30	0.2	1.0

in the absence of long-range interactions, for instance in the presence of high concentrations of excess salt, the derivative $\partial \ln s/\partial p$ equals zero and eq 25 reduces to

$$\partial p/\partial T = (\partial g/\partial \ln s)(\partial \ln s/\partial T) \qquad (29)$$

a relation that has been used previously,[20] when electrostatic polyion–polyion interactions have not been considered.

Equation 29 and related expressions are sometimes used (together with relations of the kind exemplified by eq 26) in the evaluation of the initiation parameter from experimental data.[20] This could lead to values of the initiation parameter that are too high (i.e., a low cooperativity) unless the salt concentration is high enough to suppress the dependence on the helical content in the propagation parameter, and it could also lead to an (apparent) ionic strength dependence in the cooperativity. (This is of some relevance for the molecular understanding of the transition since the cooperativity is related to the average length of a helical region in the polymer chain.) With eq 25, 26, and 29 it is possible to define an "apparent initiation parameter", emanating from the type of analysis just mentioned, as

$$(\sigma_{\text{app}})^{1/2} = \sigma^{1/2} - (m/4)[\partial(\Delta\mu_{\text{el}}/kT)\partial p] \qquad (30)$$

While the exact functional form of the above expression is limited to the conditions given in connection with eq 26, the qualitative relation between σ_{app} and σ is of general validity. (Again we stress, however, that the relation in eq 30 is only formal; the true initiation parameter, which determines the cooperativity of the transition, is taken to be constant in our treatment.)

Turning to numerical examples for carrageenan, the derivative $m(\partial g/\partial \ln s)$ equals 4.8 at the transition midpoint, as judged from the data in Figures 4 and 5. (This should be regarded as an effective value including possible effects of polydispersity.) Given this value, it is possible to calculate the "broadening factor" in eq 25:

$$\eta \equiv 1/[1 - (\partial g/\partial \ln s)(\partial \ln s/\partial p)] \qquad (31)$$

and the values of this factor for the various ι-carrageenan systems of our study are listed in Table III. It can be seen that the slope of the transition is reduced by up to 40% (for salt-free ι-carrageenan) and that the effect rapidly disappears as salt is added. One additional point is that the broadening effect is more pronounced in the presence of divalent ions than with monovalent ions if the comparison is made for conditions yielding the same transition midpoint temperature (cf. Table III). Whether or not this prediction is borne out in practice for the case of carrageenans is difficult to judge, since the effect is rather small due to the relatively low charge density of these systems and, furthermore, because of the noise level in the experimental data. However, exactly this behavior has been observed for DNA,[10] where the width of the helix-coil transition is 4–20 °C with divalent counterions and 2–10 °C with monovalent counterions. The stronger broadening in DNA is due to its higher charge density as compared to carrageenan. The more pronounced broadening with divalent counterions is perhaps somewhat unexpected; since the effect is caused by polyion–polyion interactions, it might be expected that the effect should decrease with more effectively screening ions such as divalent ions. However, such a comparison is correct only if it is made at the same ionic strength; here the comparison is instead made at salt concentrations required to induce the conformational transition at the same temperature, and it then turns out that the system with divalent ions actually has the higehst surface potential and, consequently, the strongest polyion–polyion interactions. One way to rationalize the behavior is as follows: Divalent ions are so effective in stabilizing the helix conformations that very low salt concentrations, at which the polyion–polyion interactions and the electrostatic surface potential are still quite large, will suffice. Only in the limit of very low potentials, where the interactions are determined by the ionic strength, will the effect be independent of the counterion valency.

V. Summary and Concluding Remarks

The theoretical model used here, with electrostatic interactions treated in the PBCM, gives a remarkably good description of experimental data. The salt-induced temperature shift in the conformational transition as well as the sharpening of the transition with increasing salt concentration is in quantitative agreement with experiment for ι-carrageenan in the presence of 1:1 electrolyte. As a special point is deserves mentioning that the PBCM can handle the electrostatic interactions in the presence of 0.2 M NaCl, where polyion–polyion interactions are almost negligible, *as well as* in salt-free solutions, where polyion–polyion interactions are of obvious significance. Furthermore, the conformational transition in the presence of divalent counterions is semiquantiatively predicted from NaCl data.

Turning last to the controversial question of whether the ordered conformation of the carrageenans is a double helix or a single helix (with half the charge density of the double helix), it may be noted that we here, as in our previous studies,[1,29] find that model parameters corresponding to the double helix result in good agreement with experiment. The opposite conclusion was, however, reached in a recent report on κ-carrageenan,[2] where the dependence of the transition midpoint temperature on salt concentration was analyzed in a fashion similar to that of our present work. In the former study,[2] calorimetric data taken from the literature were, in fact, claimed to support the single-helical conformation. However, we find this conclusion questionable, for the following reasons. First, the spread in the experimentally determined transition enthalpy makes any comparison with theory hazardous, as long as the reason for this spread is not well understood. Some of the calorimetric data quoted in ref 2 are actually in good agreement with the double-helical conformation, and we are not convinced that these (higher) enthalpies may be attributed to the formation of higher order aggregates, as suggested by the authors of ref 2. Second (and more importantly from a fundamental point of view), although the analysis was based on experimental data where the highly specific potassium ion was used (this ion shifts the conformational transition to much lower salt concentrations as compared to "unspecific ions"), this ion specificity was not taken into account in the theoretical treatment. Such pure electrostatic analyses of the potassium κ system, which we would regard as principally unsatisfactory, have been made also in other studies in the past,[12-17] in these cases utilizing Manning's electrostatic model. When comparing our results with those of the latter studies, one should, furthermore, be aware of the differences in theoretical transition enthalpies for κ-carrageenan, in the presence of 1:1 electrolyte, as deduced from different electrostatic models (Table II). In view of these discrepancies, as well as the experimental uncertainties just mentioned, we believe that evidence presented previously regarding the helix conformation of κ-carrageenan, based on electrostatical/thermodynamical analyses along the lines just discussed, should not be regarded as conclusive. It may be noted that the evidence presented[12,15,16] in support of the particular (extended) single-helical structure under discussion is based entirely on such analyses.

As regards the compatibility of the single-helix structure with the experimental findings of the present study, it is suspected that the single helix will not yield as marked *electrostatic effects on the sharpness of the conformational* transition as are seen experimentally (and given by the theory assuming a double-helical structure). However, we have chosen not to perform calculations for the single helix here but rather to content ourselves with the general conclusion that on the basis of electrostatic considerations there is no need to invoke this particular single-helix structure. The reason that we do not consider this structure further is that we find it highly improbable on the following a priori grounds: First, a helical structure is characterized by long-range order, which implies that the interresidue bond rotation energies have minima that are quite well-defined (i.e., deep and narrow). These well-defined minima generally have their origin in strongly directional, short-range forces, such as hydrogen bonds. Such stabilizing interactions (wich, in the double-helical structure, are accomplished intermolecularly) would, however, not seem possible in such an open structure as the proposed single helix: The single helix is almost fully extended, having a length per unit charge of 8.2 Å as compared to 10 Å for the coil (cf. Table I). Second, it is well established that the cooperativity of helix–coil transition is caused by the cost of entropy of forming the first turn in the helix before any stabilizing (enthalpic) factors can contribute.[20] However, in an almost fully extended structure, such as that of the proposed single helix, it is very difficult to see how the free energy of forming the first turn would differ significantly from that of the consecutive turns. As there could be only stabilizing interactions between nearest neighbors (in the primary structure), a totally noncooperative transition would, in fact, be anticipated for an extended single helix. This prediction is clearly not borne out by the experimental results.

Earlier investigators have demonstrated[18] a dependence of the helix–coil transition temperature, for ι- and κ-carrageenan, on the polysaccharide concentration. This result, which is in accord with the notion of a bimolecular transition, has been confirmed for the case of ι-carrageenan (the concentration dependence of the κ-carrageenan transition was not investigated) in the present study. Under some circumstances, however, it has been found[39-41] that the molecular weight of unsegmented carrageenan is independent of its conformational state, suggesting a unimolecular conformational transition. These seemingly conflicting experimental observations have yet to find a satisfactory explanation within any particular helical model. However, in view of the above considerations, the extended single-helical structure (which appears to be the only unimolecular helix conformation that has been considered explicitly to date) would not seem to be a likely candidate.

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