Helix–Coil Transitions of Ionic Polysaccharides Analyzed within the Poisson–Boltzmann Cell Model. 4. Effects of Site-Specific Counterion Binding

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ABSTRACT: The cation specificity of the salt-induced conformational transition of κ-carrageenan is analyzed in terms of site-specific binding of certain monovalent cations to the κ-carrageenan helix. The balance between general electrostatic interactions and the charge-regulating binding is treated self-consistently at the Poisson–Boltzmann level. According to the analysis, a quite weak specific interaction is sufficient to account for the drastic ion specificity observed experimentally. This specific interaction is of the same order of magnitude as that found for the complex formation between cations and simple mono- and disaccharides. Both thermodynamic data on the conformational transition and counterion NMR data on the binding of ions to the κ-carrageenan helix are well reproduced by the model, using a temperature-dependent binding constant as a single-fitting parameter. The possible nature of the binding site is discussed.

I. Introduction

This paper is the fourth of a series where we analyze electrostatic effects on the conformational equilibria of biopolymers using the Poisson–Boltzmann cell model (PBCM). In previous papers we have investigated the influence of factors such as the valency of the counterion,[1] the salt concentration,[2] the concentration of the polyion,[1] and the dielectric constant of the solvent.[3] As model systems for these studies, we have chosen the carrageenans,[4] a class of sulfated polysaccharides, which have the ability to undergo helix–coil transitions in solution. The carrageenans are quite suitable for these kinds of studies since they are available with a large range of charge densities and, furthermore, they do not titrate. So far, we have restricted our studies to the most charged helix-forming carrageenans, i.e., κ- and ι-carrageenan, which may easily be obtained in relatively pure form and which have well-defined primary structures with one and two charges per repeating disaccharide unit (Figure 1), respectively. In the carrageenans (as in most helix-forming polyelectrolytes), the helical conformation is electrostatically disfavored since the polyion charges are locally brought closer to each other on helix formation. This feature is captured by the PBCM, which has been found to give a qualitatively correct and a semiquantitatively accurate representation of all purely electrostatic effects on the conformational transition of carrageenans encountered so far, including some unexpected and, in part, contraintuitive phenomena.[1-3]

However, one of the most striking features of the κ-carrageenan conformational transition, the cation specificity, has not been analyzed in our previous studies. While the alkali-earth ions and certain monovalent ions (i.e., sodium, lithium, and tetramethylammonium) all seem to affect the conformational transition mainly by long-range Coulombic interactions, the helix conformation is strongly stabilized, but to different degrees, by other monovalent cations (i.e., potassium, cesium, rubidium, and ammonium).[5] The magnitude of the effect is illustrated by the fact that the concentration of "specific" monovalent ions required to induce helix formation of κ-carrageenan, at a given temperature, is lower by a factor of 10–60 (depending on the ion)[5] than the required concentration of "nonspecific" monovalent ions. In contrast, the cation specificity of the structurally very similar, ι-carrageenan (see Figure 1) is quite small and, for most purposes, insignificant.[6] The implications of these observations are that certain ions may stabilize the helical conformation of κ-carrageenan by binding to specific sites on the helix. This conclusion is also supported by counterion NMR spectra, where large shift and relaxation effects are seen only for specific ions and only in systems containing κ-carrageenan in the helical conformation,[7-11] whereas such effects are absent for ι-carrageenan free of κ-carrageenan impurities.[12] The NMR data also indicate a selectivity of the ion binding to the κ-carrageenan helix[7,8] but not to the coil.[11]

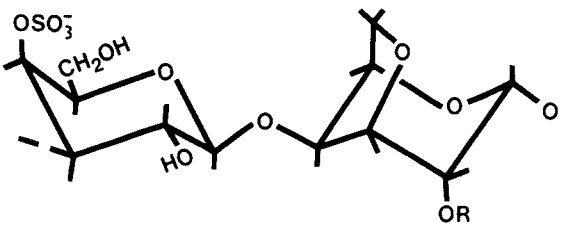

Figure 1. Repeating disaccharide structures of κ- (R = H) and ι- (R = SO_3^-) carrageenan.

If the notion of site binding of ions to the κ-carrageenan helix seems well established, very little has been known about the strength of the binding or the nature or, indeed, the number of binding sites. The purpose of this study is to analyze various data on the ion specificity of κ-carrageenan in terms of the PBCM, with the incorporation of site-specific binding. In regard to the electrostatic effects on the conformational transition, κ-carrageenan will thus, in some respects, be similar to a titrating polypeptide, and in a recent study,[13] it has been shown that the PBCM can handle the covariation in electrostatic interactions and the degree of binding for the latter types of systems. The strategy of the present study is the following. We first analyze the ion specificity of the coil–helix transition of κ-carrageenan at a given temperature, thereby gaining information about the binding constants of specific ions. Then we investigate the transition temperature vs salt concentration dependence of specific ion forms of κ-carrageenan, to extract the enthalpy of binding of the specific ions, which we compare with existing calorimetric data. Finally, we use the binding constants obtained from the thermodynamic analysis to predict the ion competition behavior observed by NMR. In the

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thermodynamic analysis we will, as previously,[1-3] assume that the temperature of onset of helix formation on cooling, T_0, reflects the helix–coil equilibrium, so that this temperature is not influenced by helix aggregation. We will also neglect possible effects of helix aggregation on the cation binding observed by NMR.

Our approach is entirely phenomenological in the sense that nothing needs to be assumed about the structure of the binding sites. In view of the experimental evidence cited above, we will here assume that only the helix conformation contains binding sites and, furthermore, that there is only one class of sites. As to the density of sites, we will consider the possibilities of one or two repeating disaccharides per site. These choices will be motivated at the end, where we speculate on the nature of the binding site and on the reason why ι- and κ-carrageenan behave so differently with respect to their site binding of ions.

II. Experimental Section

Materials. κ-Carrageenan (from *Euchema cottonii*) was obtained from Sigma Chemical Co. Two different lots, No. 124F-0604 and No. 115F-0665 (here called samples 1 and 2, respectively) with slightly differing properties were used. Segments of enhanced structural regularity were prepared as described by Bryce et al.[14] 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 cubic decimeters (M) and were obtained by weighing, assuming an ideal κ-carrageenan structure (Figure 1).

Methods. The helix–coil transition was monitored by optical rotation measured at 435 nm on a Jasco DIP-360 polarimeter in a jacketed cell with a 10-cm path length. The temperature was controlled by circulating thermostatically regulated water. As previously,[1-3] the helix onset temperature, T_0, was obtained from the sharp increase in the optical rotation observed in a cooling experiment. Isothermal conformational stability diagrams in mixed-salt systems (cf. below) show compositions of systems that are characterized by a common T_0.

III. Theoretical Model and Model Parameters

Electrostatic Model. In the Poisson–Boltzmann cell model[15-18] the solution is divided into uniform cylindrical cells, with each cell containing one polyion surrounded by water and mobile ions. The polyion is centered in the cell and is approximated as a cylindrical rod of radius a with a uniform surface charge density, σ. The radius of the cell, b, is given by the polyion concentration and, for a helix-forming polyion, by the helical content. In cylindrical symmetry, the Poisson–Boltzmann equation becomes

$$\frac{1}{r}\frac{d}{dr}\left(r\frac{d\phi}{dr}\right) = -\frac{eN_A}{\epsilon_0\epsilon_r}\sum_i c_{i0}z_i \exp(-e\phi z_i/kT), \quad a < r \le b \tag{1}$$

to be solved with the boundary conditions $d\phi/dr(b) = 0$ and $d\phi/dr(a) = -\sigma/\epsilon_0\epsilon_r$ ($\phi(b)$ is taken to be zero by convention). Here $\phi(r)$ is the electrostatic potential at a distance r from the center of the cell, c_{i0} is the concentration of mobile ions of charge z_i at $r = b$, and the other symbols carry their usual meanings.

For a polyion that possesses a number of binding sites (not necessarily equal to the number of charged groups on the polyion) that specifically bind counterions, the charge density is given by the degree of binding as

$$\sigma = \sigma_0(1 - x/n) \tag{2}$$

where σ_0 is the surface charge density in the absence of binding, x is the fraction of occupied binding sites for the conformation (helix, coil) under consideration, and n is the number of polyion charges per site. The value of x is given by the law of mass action as

$$x = \frac{K_0 \exp(-e\phi(a)/kT)c_{M,0}}{1 + K_0 \exp(-e\phi(a)/kT)c_{M,0}} \tag{3}$$

Here $c_{M,0}$ is the concentration of the binding cation at $r = b$ and K_0 is the intrinsic equilibrium constant for the binding of the ion to the site. Equations 1–3 have to be solved by an iterative procedure until the Poisson–Boltzmann equation and the law of mass action are both satisfied.

From thermodynamics, the intrinsic binding constant can be related to the standard chemical potentials[13] by

$$kT \ln K_0 = \mu_M^{0'} + n(\mu_{P,ion}^{0} - \mu_{PM}^{0}) \tag{4}$$

where

$$\mu_M^{0'} = \mu_M^{0} - kT \ln c_{solvent} \tag{5}$$

Here μ_M^0 is the standard chemical potential for the binding cation in solution, $\mu_{P,ion}^0$ is the standard chemical potential, expressed per charged group of the polyion, for a polyion with no bound cations, and μ_{PM}^0 is the corresponding standard chemical potential for a polyion with all sites occupied by binding cations.

Conformational Equilibria. The PBCM theory for the treatment of conformational equilibria of polyions has been presented previously,[1,2,13] and only a short recapitulation will be given here, together with some slight modifications that are needed for the present case. The approach is to calculate the electrostatic contribution and the contribution from specific site binding to the chemical potential per repeating unit, μ_{rep}, for each conformation of the polyelectrolyte. The chemical potential for the polyelectrolyte includes the chemical potential of the counterion; μ_{rep} is thus distinct from the chemical potential of the polyion discussed in the previous section. The repeating unit is here taken to be the disaccharide, which, for κ-carrageenan, contains one charged group. Thus, for κ-carrageenan, the chemical potentials expressed per charged group (cf. above) are the same as the chemical potentials per repeating unit.

Various equivalent expressions for μ_{rep} exist. For reasons of physical transparency, we will here, as in ref 13, explicitly write the chemical potential of the polyelectrolyte as a sum of three terms

$$\mu_{rep} = \mu_{el} + \mu_{binding} + \mu_{nonel} \tag{6}$$

where the various terms are defined as

$$\mu_{nonel} = \mu_{P,ion}^{0} \tag{7}$$

$$\mu_{binding} = (kT/n)\{x \ln (x) + (1 - x) \ln (1 - x) - x \ln (K_0 c_{M,0})\} + 1/n\{\mu_M^{0'} + kT \ln c_{M,0}\} \tag{8}$$

and

$$\mu_{el} = -e\phi(a)\,(1 - x/n) - E_{el} + kTV_{solvent}N_A\sum(c_{i,av} - c_{i0}) \tag{9}$$

In eq 9, $V_{solvent}$ is the volume of solvent (per repeating unit) in the cell, $c_{i,av}$ the cell average concentration of mobile ionic species i in the cell, and E_{el} the electrostatic interaction energy, defined as

$$E_{el} = (\epsilon_r\epsilon_0/2)\int(\nabla\phi)^2\,dV \tag{10}$$

As defined above, μ_{nonel} is the nonelectrostatic chemical potential for a repeating unit without a site-bound cation,

whereas μ_{el} represents the purely electrostatic contribution to μ_{rep} at equilibrium binding of specific counterions, when the average charge of a repeating unit equals $-(1 - x/n)$. This definition of μ_{el} is consistent with the expression derived previously for mixtures of charged and uncharged units.[18] Finally, $\mu_{binding}$ is the contribution due to specific binding, which, as defined above, also contains the chemical potential of the specific cation (the second bracketed term on the right-hand side of eq 8). The terms within the first brackets on the right-hand side of eq 8 may also be given simple physical interpretations: The first two terms represent the (ideal) entropy of mixing occupied and unoccupied binding sites, whereas the third term represents the intrinsic free energy (at equilibrium degree of binding) associated with transferring binding ions from solution (at activity = $c_{M,0}$) to the bound state.

Equations 7–9 differ slightly from the corresponding eqs 20 and 21 of ref 13, but the derivation is quite analogous. In part, the differences are due to different choices of reference state. The changes in the chemical potential are taken relative to the nonelectrostatic free energy for a repeating unit, μ_{nonel}, which here is defined as the standard chemical potential for a unit *without* a site-bound cation. The reason for this choice is that it is convenient to have analogous reference states for both conformations, and in the present case only one conformation (i.e., the helix) carries binding sites. The choice of reference state only affects the way that the standard chemical potentials, $\mu_{P,ion}^0$ and μ_{PM}^0 are included in the contributions μ_{nonel} and $\mu_{binding}$; the total chemical potential, μ_{rep}, is, however, not affected. (The addition of the difference $\mu_{P,ion}^0 - \mu_{PM}^0$ to μ_{nonel} and the subtraction of the same quantity from $\mu_{binding}$ of ref 13 leads, with the use of eq 4, to the present result.)

Another source of the differences between eqs 7–9 and the previous results is that we here allow the number of repeating units per site to differ from unity. This affects the way that the charge density depends on the degree of binding and, also, the entropy of mixing between occupied and unoccupied sites, which, expressed per disaccharide, will scale with the number of disaccharides that form the site. The incorporation of these modifications into the derivations of refs 13 and 18 is straightforward, and we have therefore only cited the results.

The binding contribution to the chemical potential for a nonbonding conformation (i.e., the coil) is obtained by taking the limit $K_0 \to 0$, which yields

$$\mu_{binding}(K_0=0) = 1/n\{\mu_M^{0'} + kT \ln c_{M,0}\} \quad (11)$$

Thus, with the definition adopted here, $\mu_{binding}$ is finite (and equal to the chemical potential of the binding cation) also when the binding constant is zero. Note, however, that since this nonvanishing contribution is equal for the coil and the helix, it does not contribute to the *difference* in chemical potential between the two conformations, $\Delta\mu_{rep}$. The latter difference may now be obtained as

$$\Delta\mu_{rep} = \mu_{rep}(\text{coil}) - \mu_{rep}(\text{helix}) \quad (12)$$

with the various contributions

$$\Delta\mu_{el} = \mu_{el}(\text{coil}) - \mu_{el}(\text{helix}) \quad (13)$$

$$\Delta\mu_{binding} = \mu_{binding}(\text{coil}) - \mu_{binding}(\text{helix}) = -(kT/n)\{x \ln (x) + (1-x) \ln (1-x) - x \ln K_{0,helix}c_{M,0}\} \quad (14)$$

Table I
Model Parameters for κ-Carrageenan

coil		helix	
l, Å	a, Å	l, Å	a, Å
10	3.3	4.1	5.1

and

$$\Delta\mu_{nonel} = \mu_{P,ion}^0(\text{coil}) - \mu_{P,ion}^0(\text{helix}) \quad (15)$$

The above definition of $\Delta\mu_{nonel}$ is consistent with that used in previous parts of this series where the standard chemical potentials were never expressed explicitly. As previously,[2,3] we will here assume that $\Delta\mu_{nonel}$ contains one enthalpic and one entropic part, both of which are independent of the temperature or the ionic content of the system.

It deserves pointing out what when the binding constant for the helix is zero, i.e., when there is no specific binding to either conformation, $\Delta\mu_{binding}$ vanishes and the expressions for $\Delta\mu_{el}$ and $\Delta\mu_{nonel}$ (eqs 13 and 15) become identical with the expressions used previously to treat conformational equilibria in the absence of specific ion binding. Another special case of interest is when the degree of binding is not influenced by electrostatic interactions, i.e., when either the electrostatic surface potential or the charge of the binding species is zero. Equation 3 then reduces to the ordinary Langmuir-type isotherm, and the expression of $\Delta\mu_{binding}$ can be rewritten in the more compact form

$$\Delta\mu_{binding} = (kT/n) \ln (1 + K_{0,helix}c_{M,0}) \quad (16)$$

Equation 16 may be recognized as the expression predicted by Schellman's analysis[19] of the effects of ligand binding on the conformational equilibria of uncharged macromolecules.

The chemical potential, μ_{rep}, does not include the entropy of mixing between the two conformational states, which must be evaluated in some suitable model. In the Zimm–Bragg model for helix–coil transitions,[20] the statistical weights are given by the so-called helix initiation and propagation parameters, and the entropy of mixing is introduced by an ensemble summation. The propagation parameter, s, of the Zimm–Bragg model is related to the chemical potential difference per repeating unit derived above via the expression

$$kT \ln s = \Delta\mu_{rep} \quad (17)$$

If the helix initiation parameter is independent of temperature or salt content of the system (a standard assumption that we will also make here), the helical content of the system is uniquely defined by the value of the propagation parameter and, thus, by the value of $\Delta\mu_{rep}/kT$.

Model Parameters. To perform calculations within the above model, it is necessary to specify the dimensions of the model rod for each conformation, i.e., the length per polyion charge, l, and the radius, a. These parameters are listed in Table I and are the same as those that have been used previously.[1-3] This means that we assume a stretched-out coil conformation and a double-helical conformation with dimensions inferred from X-ray data[21,22] on oriented fibers. The thickness of each model rod has been chosen so as to yield a specific volume per disaccharide in agreement with experimental data.[23] An iterative scheme for solving the Poisson–Boltzmann cell model for finite concentrations of all species (including the polyion) is given in ref 1.

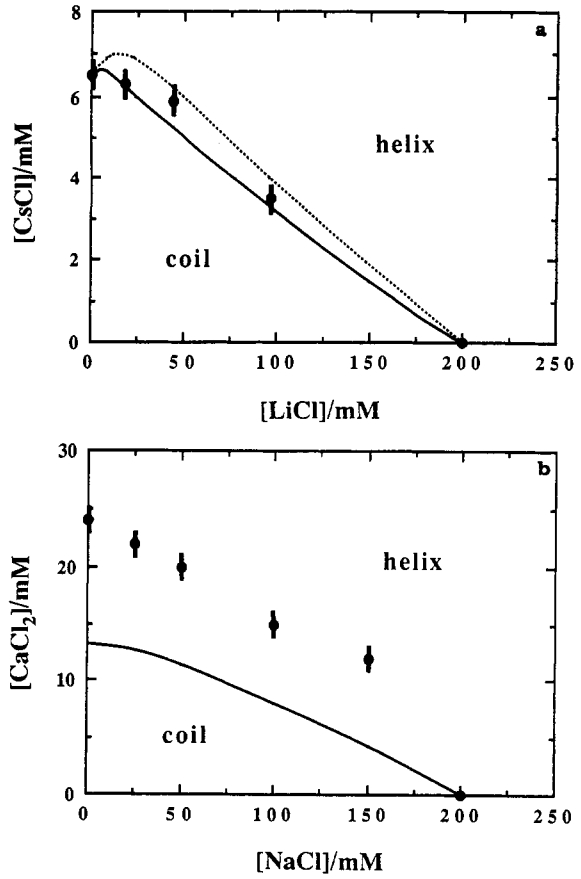

Figure 2. Conformational stability diagrams (see text) for κ-carrageenan (sample 1) in mixed electrolyte solutions: (a) 3.8 mM carrageenan in LiCl/CsCl at 17 °C. The molar ratios of the cesium and lithium forms of carrageenan are the same as the molar ratios of the salts. Points indicates experimental data; lines are model predictions (see text) for $n = 1$, $K_{0,helix} = 1.86$ M^{-1} (dotted line) and for $n = 2$, $K_{0,helix} = 5.0$ M^{-1} (solid line). (b) 5 mM carrageenan in NaCl/$CaCl_2$ at 18 °C. The solid line is calculated (see text) for purely electrostatic polyion–counterion interactions.

IV. Results and Discussion

Conformational Transitions. Isothermal Data. In Figure 2a the drastic effect of cesium (a specific ion) on the conformational equilibrium of κ-carrageenan is presented in the form of a stability diagram. The diagram shows the concentration of cesium required for onset of helix formation at various concentrations of lithium (a nonspecific ion) and at a fixed temperature. The salt concentrations in the pure ion forms differ by a factor of 30, being 6.5 and 200 mM for cesium and lithium, respectively. For comparison, a stability diagram for mixtures of nonspecific monovalent and divalent counterions (sodium and calcium, respectively; data taken from ref 1), obtained under closely similar conditions, is given in Figure 2b. For the pure calcium form, the calcium concentration required for helix formation is 24 mM. Thus, on a molar basis, cesium is even more efficient than a divalent counterion in stabilizing the more highly charged helix conformation, which clearly demonstrates the importance of specific interactions.

The theoretical curves in Figure 2a were calculated by using the requirement that the sum $\Delta\mu_{el} + \Delta\mu_{binding}$ must be constant along the transition curve. This follows from the isothermal conditions and the assumptions that neither the cooperativity of the transition nor the nonelectrostatic chemical potential difference varies with the salt content of the system. The pure lithium system was taken as the reference point (i.e., the sum $\Delta\mu_{el} + \Delta\mu_{binding}$ along the theoretical curves equals $\Delta\mu_{el}$ calculated for the pure lithium system), and the intrinsic binding constant for cesium was chosen so as to yield agreement with the experimental data for the pure cesium system. Two theoretical curves are shown, corresponding to our two choices of the density of binding sites on the carrageenan helix, and both curves are in good agreement with the experimental data. In either case, the analysis indicates a quite weak interaction on the molecular level ($1 < K_{0,helix}/M^{-1} < 5$), despite the large macroscopic effect of the ion specificity. This large effect is caused by the electrostatic enhancement of the concentration of counterions near the polyion (cf. the binding isotherm, eq 3), which leads to a large degree of binding. Thus, for the pure cesium system of Figure 2a, we obtain $x = 0.38$ ($n = 1$) or $x = 0.67$ ($n = 2$). For comparison, $x = 0.05$ for the hypothetical uncharged molecule with $K_0 = 5.0$ M^{-1} and $n = 2$ under the same conditions of cesium and macromolecular concentrations. The large degree of binding to the helix makes $\Delta\mu_{el}$ much less unfavorable for the helix conformation in the presence of specific cations, an in the present case, it is this effect (rather than the contribution from $\Delta\mu_{binding}$) that accounts for the helix-stabilizing effect of the cesium ions in the present case.

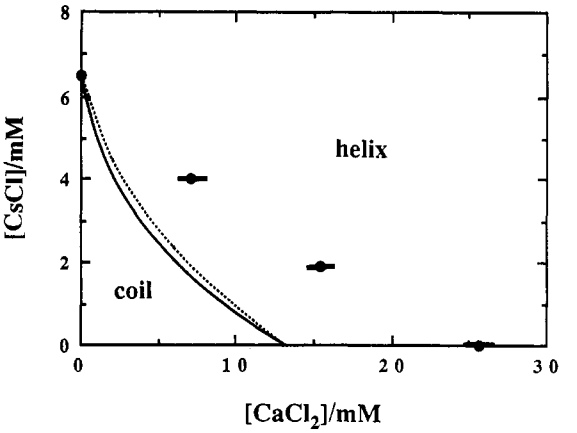

Figure 3. As Figure 2, but for 3.8 mM carrageenan (sample 2) at 21 °C in $CaCl_2$/CsCl mixtures. (The slightly higher temperature as compared to Figure 2 possibly reflects a somewhat higher molecular weight for sample 2 than for sample 1.)

In order to further study the balance between general electrostatic interactions and site-specific ion binding, we also made a stability diagram for mixtures of CsCl and $CaCl_2$ (Figure 3). Compared to the stability diagram for the Ca/Na mixture (Figure 2b), the diagram in Figure 3 shows a new feature in that the stability boundary is concave rather than convex. In the absence of specific interactions, both the theoretical and the experimental stability diagrams with mixed mono- and divalent ions are always convex (sometimes even with a maximum).[1,3] It is therefore gratifying that the qualitative difference in the curvature between the Na/Ca and Cs/Ca mixtures is predicted by the theoretical model. Note that, since all theoretical parameters are defined from the fits in Figure 2a, there are no free parameters in the theoretical curves of Figure 3. Consequently, we find in Figure 3, as in Figure 2b, that the theoretically predicted concentrations of $CaCl_2$ are too low by a factor or almost 2. One possible source of this model deficiency could be the neglect of charge discreteness.

Conformational Transitions. Salt Concentration Dependence. Data on the salt concentration dependence of the coil–helix transition midpoint temperature, T_m, of κ-carrageenan in various salts have been presented by Rochas and Rinaudo (Figure 7 of ref 5). For each

Table II
Thermodynamic Interaction Parameters for Specific Ions

	$K_{0,helix}$ (M^{-1}) at 290 K		$\Delta H^0_{binding}$, kJ/mol		ΔH_{cal},[a] kJ/mol	
ion	$n = 1$	$n = 2$	$n = 1$	$n = 2$	$n = 1$	$n = 2$
Rb^+	3.42	9.85	24.5	24.6	23.0	21.6
Cs^+	1.86	5.0	23.1	24.4	21.5	20.4

[a] Refers to pure salt forms.

investigated salt, a linear relation between $1/T_m$ and the logarithm of the "total ionic concentration", C_T, was found. To theoretically predict such data, we need thermodynamic information that is not given by the PBCM. As was pointed out above, within the assumptions of our model, a given helical content corresponds to a unique value of the propagation parameter. In the present case this leads to the condition[2]

$$(\Delta\mu_{el}/kT)_{p=constant} + (\Delta\mu_{binding}/kT)_{p=constant} + (\Delta H_{nonel}/kT)_{p=constant} = constant \quad (18)$$

where p is the degree of conversion from coil to helix, T is the temperature that gives the (arbitrarily) specified helical content at various salt concentrations and ΔH_{nonel} is the nonelectrostatic enthalpy of the transition. To arrive at eq 18, we have also made use of our assumption that the nonelectrostatic entropy of the transition is independent of temperature and salt content. Thus, the calculation of the temperature shift in a transition curve on the addition of specific ions requires knowledge of ΔH_{nonel} and of the temperature dependence in $K_{0,helix}$, the intrinsic binding constant. Our previous analysis[2] of the salt dependence of the transition in nonspecific 1:1 electrolytes yielded ΔH_{nonel} = 12.7 kJ/mol of repeating units, a value that we will adopt also here, but the temperature dependence in $K_{0,helix}$ is not known. We have therefore used our theory to evaluate the latter temperature dependence from the data for specific ions of Rochas and Rinaudo: A few points covering the experimental range of the $1/T_m$ vs C_T curves of Figure 7 of ref 5 were selected, and, for each point, calculations were made where the value of $K_{0,helix}$ was adjusted so as to give agreement between theory and experiment. In these calculations, we assumed infinitely dilute systems with ionic activities equaling C_T. The temperature dependence of the binding constants thus obtained exhibited normal Arrhenius behavior, and from this an intrinsic enthalpy of binding ($\Delta H^0_{binding}$) in the range 23–24 kJ/mol could be obtained. Binding enthalpies and binding constants at 290 K for the cesium and rubidium ions and for the two choices of site densities are given in Table II.

The intrinsic enthalpy of binding should be possible to detect calorimetrically. To a good approximation (see the Appendix), the calorimetric transition enthalpy depends on the nonelectrostatic transition enthalpy, the intrinsic enthalpy of binding, and the degree of site binding according to the relation

$$\Delta H_{cal} \approx \Delta H_{nonel} + (x/n)\Delta H^0_{binding} \quad (19)$$

One curious result of our analysis of the data of Rochas and Rinaudo is that the degree of binding along the specific curves is almost constant in the concentration range considered (6–100 mM of salt). This means that the effect of an increase in the salt concentration is compensated by the decrease in the binding constant at higher temperatures. As a consequence, the transition enthalpy is almost independent of the salt concentration for any given pure salt form. For the pure salt forms involving the specific ions potassium, rubidium, and cesium, the transition enthalpy becomes 20–23 kJ/mol compared to 12.7 kJ/mol for nonspecific salts (Table II). With mixed cations it should be possible to obtain a range of values between 12.7 and 23 kJ/mol. Unfortunately, the spread in the experimental values of the calorimetric transition enthalpy reported in the literature (3.5–24.5 kJ/mol, depending on system conditions and experimental procedure,[9,24-31]) is so large that it cannot be said with certainty if this prediction is borne out in practice. It is true that the only study concerned with nonspecific counterions[31] also produced the lowest set of values recorded (3.5–10.4 kJ/mol), but also these data show a considerable spread, and their interpretation is complicated by the fact that they, in part, pertain to systems where specifically helix-promoting anions are present (cf. below).

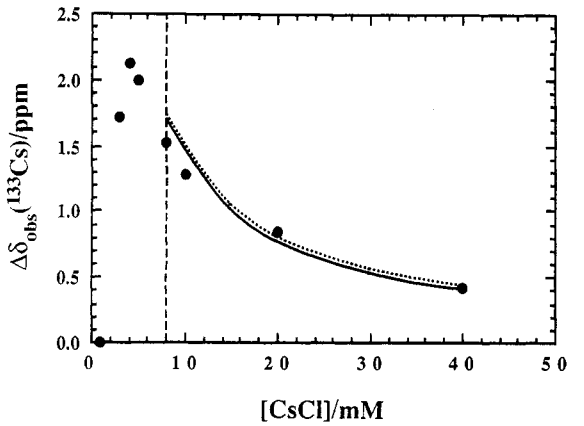

Figure 4. Variation in ^{133}Cs NMR shift with concentration of CsCl added to 1 mM tetramethylammonium-κ-carrageenan at 10 °C. Experimental data are from ref 9. The dashed vertical line indicates the CsCl concentration where full conversion to helix has been achieved. Lines are calculated for $n = 1$, $\Delta\delta_b$ = 36.4 ppm and $K_{0,helix}$ = 2.4 M^{-1} (dotted) and for $n = 2$, $\Delta\delta_b$ = 41.5 ppm and $K_{0,helix}$ = 6.4 M^{-1} (solid).

Ion Binding from Counterion NMR Shifts. With the binding constants obtained from the thermodynamic analysis above, it is possible to predict the degree of ion binding in κ-carrageenan systems. The observed chemical shifts in the NMR of a specifically binding ion, such as $^{133}Cs^+$, are directly related to the degree of binding. If a rapid exchange between free (f) and site-bound (b) ions is assumed, the observed chemical shift (relative to an arbitrary reference frequency), δ_{obs}, is given by

$$\delta_{obs} = p_b\delta_b + (1 - p_b)\delta_f \quad (20)$$

where δ_f and δ_b are the intrinsic shifts of the free and bound ions, respectively, and p_b is the fraction of bound ions, which can be calculated from the binding isotherm (eq 3) in the theoretical section. If one further assumes that the intrinsic shift for free ions is the same as that in a reference solution with only salt present, the observed shift relative to the reference solution, $\Delta\delta_{obs}$, becomes

$$\Delta\delta_{obs} = p_b(\delta_b - \delta_f) \equiv p_b\Delta\delta_b \quad (21)$$

Since the binding constants for the two site densities (one or two repeating units per site) considered here have been defined from thermodynamic data, the only free parameter is $\Delta\delta_b$, which we will here assume to be independent of temperature and salt concentration.

Figures 4 and 5 show experimental data (from studies[7-9] by Grasdalen and co-workers) on the salt dependence of the $^{133}Cs^+$ shifts in κ-carrageenan solutions, together with the predictions of our model. The initial increase seen in most experimental data is due to the salt-induced coil-to-helix transition. The calculations refer to all-helical

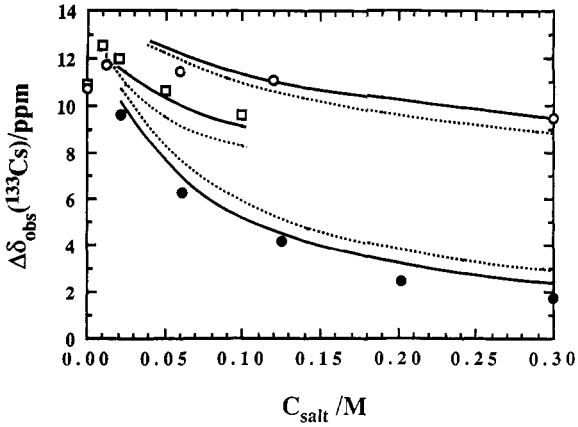

Figure 5. Variation in ^{133}Cs NMR shift with concentration of NaCl (○), $CaCl_2$ (□), or CsCl (●) added to 48 mM Cs-κ-carrageenan at 25 °C. Experimental data are from ref 8. Lines are calculated for $n = 1$, $\Delta\delta_b = 36.4$ ppm and $K_{0,helix} = 1.6\ M^{-1}$ (dotted) and for $n = 2$, $\Delta\delta_b = 41.5$ ppm and $K_{0,helix} = 4.1\ M^{-1}$ (solid).

systems. It should be pointed out that the data in Figures 4 and 5 cover quite a wide range of conditions with regard to the electrostatic surface potential and the degree of cesium binding. The good agreement between experiment and theory is therefore gratifying. In particular, the calculations for $n = 2$ give a very good representation of the data. The decrease in cesium binding when inert cations like sodium or calcium (Figure 5) are added is correctly predicted by the model. This effect is not due to any competition of inert cations with cesium for the binding sites. It is a purely electrostatic effect, caused by the reduced attraction between the cations and the polysaccharide as the ionic strength is increased.

The values of the fitted intrinsic shifts are 36.4 ppm for $n = 1$ and 41.5 ppm for $n = 2$. These shifts may be compared to the intrinsic shifts reported for $^{133}Cs^+$ in complexes with various crown ethers and cryptands, which range from −50 ppm to +245 ppm (with the present sign convention).[32] The largest experimental shift that has been observed for cesium in κ-carrageenan is 46 ppm in a salt-free 6% sample with a mixture of cesium and tetramethylammonium counterions.[9] In the same study, the intrinsic shift was estimated (neglecting electrostatic effects) to 55 ppm. The intrinsic shift must be larger than the largest shift observed experimentally, and the low value of our fitted intrinsic shift thus indicates that our model somewhat overestimates the number of bound ions. Inasmuch as our basic assumptions regarding the specific ion binding are correct, this would imply that the binding constants extracted from the thermodynamic analysis above are slightly too large. On the other hand, quantitatively accurate estimates of the binding constants should not be expected, considering the fact that for nonspecific ions, the agreement between the model and experiment was only semiquantitative (cf. Figure 2b and refs 1 and 2).

Nature of the Binding Site. The treatment so far has been phenomenological in the sense that the theoretical model assumes nothing about the exact location or structure of the site. Since the model assumes a uniform surface charge, it does not distinguish between cases when the binding is directly to a charged group with charge annihilation (such as for the binding of a proton to a carboxylate group) or when it is to some other site on the polyion, where the charged group is not involved at all. In the present case, there seems to be no unequivocal experimental information on the location of the site. Although IR spectra of various ion forms of carrageenans have been taken to suggest that the sulfate group is involved in the binding site,[30,33] this interpretation has been challenged in later work.[34] Also, there seems to be no a priori reason to assume that the sulfate group has any special role for the site binding of cations apart from creating an electrostatic surface potential, as the sulfate group as such is not recognized as a particularly good ligand of alkali ions. Moreover, if the sulfate group were, in fact, involved, one might expect that the conformational transition of ι-carrageenan, which has one sulfate group more than κ-carrageenan, should exhibit ion specificity, contrary to experimental evidence.

As ether oxygens and hydroxy groups are known to be good ligands to cations, it seems natural to assume that the binding site consists of such functions on the κ-carrageenan helix. The complex formation between cations and simple uncharged mono- and disaccharides in aqueous solution is a well-studied phenomenon, and such complexes usually involve at least three sugar hydroxy groups or ether oxygens, arranged so that the oxygen atoms can coordinate the cation and replace some of the hydration shell.[35] The binding constants reported for different saccharides vary within the range 0.1–10 M^{-1} (with the higher binding constants reported for the divalent alkaline-earth ions), which covers the binding constants obtained here. Data on the intrinsic enthalpy of the binding are scarce, but for the binding of calcium ions to methyl glycofuranosides[36] and to D-ribose,[37] the enthalpies are on the order of 10 kJ/mol, which again compares well with the data in Table II.

From what may be inferred from the studies of simple sugars,[35] the κ-carrageenan coil is not expected to exhibit any significant cation specificity, since its primary structure does not contain a suitable geometrical arrangement of sugar oxygen atoms (see Figure 1). However, the situation is different for the double helix, where two chains may contribute oxygen functions to a site. The cation selectivity of the κ-carrageenan helix differs from that of simple saccharides, since the divalent alkaline-earth ions interact nonspecifically with κ-carrageenan. On the other hand, the same selectivity patterns as in κ-carrageenan have been found for macrocyclic compounds containing oxygen functions, i.e., the crown ethers and cryptands.[38] For the latter compounds, it is found that the size selectivity of the binding increases with decreasing flexibility of the conformation of the ligand. As the formation of a double helix puts strong restrictions on the conformations of the individual chain, a site in the double helix of κ-carrageenan may indeed be expected to be quite rigid and, therefore, size selective.

At present, a search for such possible sites is hampered by the lack of detail in the available structural data on the κ-carrageenan helix.[21,22] In their recent analysis of X-ray diffractograms obtained from oriented fibers, Millane et al.[22] seem to favor a duplex of parallel chains, which are offset from a half-staggered arrangement, but some antiparallel duplex models could not be excluded. If we believe that oxygen atoms from two chains are involved in the site, the former model would imply that each site requires two disaccharides. On the other hand, in the antiparallel case, both $n = 2$ and $n = 1$ would be possible, depending on whether or not the site involves equivalent oxygen atoms from the different chains. Although our model calculations for two repeating units per site give a somewhat better agreement with the experimental results (Figures 4 and 5), we do not regard the discrepancies for $n = 1$ to be sufficiently large to rule out the latter alternative.

Without detailed knowledge of the location of the site on the κ-carrageenan helix, one can, of course, only speculate on the reason why the site is absent in ι-carrageenan. An obvious possibility is that it is due directly to the difference in primary structure between the polysaccharides. Thus, if the hydroxyl group on carbon 2 of the anhydrogalactose unit is involved in the binding site, the site should disappear on replacement of this hydroxyl group with a sulfate group in ι-carrageenan. Other possibilities are related to the differences in secondary structure (helix conformation) between the two polysaccharides. According to X-ray evidence, the ι-carrageenan helix is a duplex with parallel chains in an exactly half-staggered arrangement. In the parallel-chain alternative proposed for κ-carrageenan,[22] the chains are offset from the half-staggered arrangement, a difference that in itself might result in the creation of a site. Last, if the κ-carrageenan helix is antiparallel, the relative positions of oxygen atoms from the two chains is altogether different from those in ι-carrageenan.

Concluding Remarks. The strength of the above analysis lies in its ability to provide a unified explanation of quite independent experimental observations for the κ-carrageenan system (i.e., the ion specificity of the conformational transition and the salt-dependent NMR shifts of specific counterions) in terms of a single parameter (i.e., the intrinsic equilibrium constant for the specific binding of certain ions to the κ-carrageenan helix) that is found to have a value within the ranges expected from comparable systems. The analysis shows that even with a comparatively weak interaction between a specific ion and the κ-carrageenan helix, the degree of binding is substantial even at low average concentrations of cesium. This means that both the intrinsic enthalpy of the ion binding and the effect of the ion binding on the charge density of the polyion must be included in the thermodynamic analysis of the effects of specific ions on the conformational transition of κ-carrageenan. In previous such analyses,[9,25-28,39,40] both these effects have been neglected.

In the context of this article, we should also briefly touch upon the observed anion specificity[31,41] of the conformational transition of κ-carrageenan. (Note that this specificity is considerably less strong than the cation specificity.) In our opinion, the available experimental evidence suggests that this specificity is due to a (weak) binding of certain anions to the κ-carrageenan helix. Such a binding could explain why these anions stabilize the helical state also relative to the aggregated state, where the number of exposed anion-binding sites presumably decrease. It would also explain the observation that the calorimetric enthalpy of the transition increases in magnitude in the presence of the helix-promoting anions[31] and, also, the large broadening of the NMR line widths of helix-stabilizing anions that accompanies helix formation.[31,41] Although the theoretical analysis of these effects, along the lines of this work, is straightforward, we leave it to a future occasion when more experimental data on the anion specificity is available.

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Appendix

The transition enthalpy is related to the chemical potential differences by the expression

$$\Delta H_{cal} = -T^2\partial(\Delta\mu_{rep}/T)\partial T = -T^2[\partial(\Delta\mu_{el}/T)/\partial T + \partial(\Delta\mu_{binding}/T)/\partial T + \partial(\Delta\mu_{nonel}/T)/\partial T] \quad (A1)$$

As shown by Marcus,[17] the differentiation with respect to temperature can be performed at a constant degree of binding (x = constant) despite the fact that the binding is temperature dependent. The three derivatives in the last member of eq A1 can therefore be evaluated separately. Starting from left to right, we obtain

$$\Delta H_{el} = -T^2[\partial(\Delta\mu_{el}/T)/\partial T] = \Delta E_{el}(1 + (T/\epsilon_r)\,\partial\epsilon_r/\partial T) \approx -0.36\Delta E_{el} \text{ (at 25 °C in } H_2O) \quad (A2)$$

$$\Delta H_{binding} = -T^2[\partial(\Delta\mu_{binding}/T)/\partial T] = -T^2\partial[(k/n)x \ln K_{0,helix}]\partial T = (x/n)\Delta H^0_{binding} \quad (A3)$$

(where the least two equalities in eq A3 follow as a consequence of eqs 14 and 4) and

$$\Delta H_{nonel} = -T^2[\partial(\Delta\mu_{nonel}/T)/\partial T] \quad (A4)$$

which finally gives the calorimetric transition enthalpy per repeating unit

$$\Delta H_{cal} = -0.36\Delta E_{el} + \Delta H_{nonel} + (x/n)\Delta H^0_{binding} \approx \Delta H_{nonel} + (x/n)\Delta H^0_{binding} \quad (A5)$$

ΔE_{el} can be neglected compared to the other quantities in eq A5 since it is on the order of 0.1–0.4 kJ/mol for the system under consideration. ΔH_{nonel} was previously found to be 12.7 kJ/mol.

References and Notes

(1) Nilsson, S.; Piculell, L.; Jönsson, B. *Macromolecules* **1989**, *22*, 2367.
(2) Nilsson, S.; Piculell, L. *Macromolecules* **1989**, *22*, 3011.
(3) Nilsson, S.; Piculell, L. *Macromolecules* **1990**, *23*, 2776.
(4) Rees, D. A.; Morris, E. R.; Thom, D.; Madden, J. K. In *The Polysaccharides*; Aspinall, G. O., Ed.; Academic Press: New York, 1982; Vol. 1, pp 195–290.
(5) Rochas, C.; Rinaudo, M. *Biopolymers* **1980**, *19*, 1675.
(6) Piculell, L.; Håakansson, C.; Nilsson, S. *Int. J. Biol. Macromol.* **1987**, *9*, 297.
(7) Grasdalen, H.; Smidsrød, O. *Macromolecules* **1981**, *14*, 229.
(8) Smidsrød, O.; Grasdalen, H. *Hydrobiologia* **1984**, *116/117*, 19.
(9) Paoletti, S.; Delben, F.; Cesàro, A.; Grasdalen, H. *Macromolecules* **1985**, *18*, 1834.
(10) Belton, P. S.; Morris, V. J.; Tanner, S. F. *Int. J. Biol. Macromol.* **1985**, *7*, 53.
(11) Piculell, L.; Nilsson, S.; Ström, P. *Carbohydr. Res.* **1989**, *188*, 121.
(12) Piculell, L.; Rochas, C. *Carbohydr. Res.* **1990**, *208*, 127.
(13) Nilsson, S.; Zhang, W. *Macromolecules* **1990**, *23*, 5234.
(14) Bryce, T. A.; Clark, A. H.; Rees, D. A.; Reid, D. S. *Eur. J. Biochem.* **1982**, *122*, 63.
(15) Fuoss, R. M.; Katchalsky, A.; Lifson, S. *Proc. Natl. Acad. Sci.* **1951**, *37*, 579.
(16) Alfrey, T.; Berg, P. W.; Morawetz, H. *J. Polym. Sci.* **1951**, *7*, 543.
(17) Marcus, R. A. *J. Chem. Phys.* **1955**, *23*, 1057.
(18) Jönsson, B.; Wennerström, H. *J. Phys. Chem.* **1987**, *91*, 338.
(19) Schellman, J. A. *Biopolymers* **1975**, *14*, 999.
(20) Zimm, B. H.; Bragg, J. K. *J. Chem. Phys.* **1959**, *31*, 526.
(21) Anderson, N. S.; Campbell, J. W.; Harding, M. M.; Rees, D. A.; Samuel, J. W. B. *J. Mol. Biol.* **1969**, *45*, 85.

(22) Millane, R. P.; Chandrasekaran, R.; Arnott, S.; Dea, I. C. M. *Carbohydr. Res.* **1988**, *182*, 1.
(23) Gekko, K.; Mugishima, H.; Koga, S. *Int. J. Biol. Macromol.* **1985**, *7*, 57.
(24) Snoeren, T. H. M.; Payens, T. A. J. *Biochim. Biophys. Acta* **1976**, *437*, 264.
(25) Rochas, C.; Rinaudo, M. *Carbohydr. Res.* **1982**, *105*, 227.
(26) Rochas, C.; Mazet, J. *Biopolymers* **1984**, *23*, 2825.
(27) Rochas, C. *J. Therm. Anal.* **1985**, *30*, 1375.
(28) Cesaro, A.; Delben, F.; Paoletti, S.; Scagnolari, F. *Termochim. Acta* **1985**, *85*, 465.
(29) Morris, E. R.; Rees, D. A.; Norton, I. T.; Goodall, D. M. *Carbohydr. Res.* **1980**, *80*, 317.
(30) Norton, I. T.; Goodall, D. M.; Morris, E. R.; Rees, D. A. *J. Chem. Soc., Faraday Trans. 1* **1983**, *79*, 2475.
(31) Norton, I. T.; Morris, E. R.; Rees, D. A. *Carbohydr. Res.* **1984**, *134*, 89.
(32) Mei, E.; Popov, A. I.; Dye, J. L. *J. Am. Chem. Soc.* **1977**, *99*, 6532.
(33) Belton, P. S.; Wilson, R. H.; Chenery, D. H. *Int. J. Biol. Macromol.* **1986**, *8*, 247.
(34) Belton, P. S.; Goodfellow, B. J.; Wilson, R. H. *Macromolecules* **1989**, *22*, 1636.
(35) Angyal, S. J. *Adv. Carbohydr. Chem. Biochem.* **1989**, *47*, 1.
(36) Vesala, A.; Lönnberg, H. *Acta Chem. Scand., Ser. A* **1981**, *35*, 123.
(37) Morel, J.-P.; Lhermet, C.; Morel-Desrosiers, N. *Can. J. Chem.* **1986**, *64*, 996.
(38) Lamb, J. D.; Reed, M. I.; Christensen, J. J.; Eatough, D. J. In *Coordination Chemistry of Macrocyclic Compounds*; Melson, G. O., Ed.; Plenum Press: New York, 1979; pp 145–217.
(39) Paoletti, S.; Smidsrød, O.; Grasdalen, H. *Biopolymers* **1984**, *23*, 1771.
(40) Jamnik, A.; Bratko, D. *Vestn. Slov. Kem. Drus.* **1987**, *34*, 15.
(41) Grasdalen, H.; Smidsrød, O. *Macromolecules* **1981**, *14*, 1842.

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