Macromolecules **1990,** *23,* 2776–2780

Helix–Coil Transitions of Ionic Polysaccharides Analyzed within the Poisson–Boltzmann Cell Model. 3. Solvent Effects

Svante Nilsson* and Lennart Piculell

Physical Chemistry 1, University of Lund, Chemical Center, Box 124, S-22100 Lund, Sweden

Received March 7, 1989; Revised Manuscript Received December 1, 1989

ABSTRACT: The effect of mixed mono- and divalent counterions on the helix–coil transition of κ- and ι-carrageenan (two charged polysaccharides) in formamide has been studied experimentally and theoretically, and the results are compared with our previously reported results obtained with water as the solvent. In either solvent, the addition of 1:1 electrolyte to a system containing divalent counterions may either stabilize or destabilize the helix conformation, depending on the exact conditions. However, this destabilizing effect appears in a more narrow range of salt concentrations in formamide as compared to the aqueous case. Furthermore, the ability of divalent cations to promote helix formation at comparatively low concentrations is less pronounced in formamide than in water. Theoretically, the electrostatic interactions are analyzed within the full Poisson–Boltzmann equation in a cylindrical cell model, and it is found that this model can explain the differences in the salt effects in the two solvents in a semiquantitative way by only taking their differing dielectric constants into account. In addition to the above purely electrostatic effects, some new observations regarding the cation specificity of the carrageenan helix–coil transition in dimethyl sulfoxide and formamide are reported.

I. Introduction

Electrostatic interactions have an important role in the conformational equilibria of charged biopolymers. In two previous papers[1,2] (henceforth referred to as part 1 and part 2, respectively) we have analyzed electrostatic effects on the helix–coil equilibria of carrageenans[3] by calculating the electrostatic contribution to the propagation parameter in the Zimm–Bragg model of helix–coil transitions[4] using the full Poisson–Boltzmann equation in a cell model[5–7] (the so-called Poisson–Boltzmann cell model, or PBCM). Whereas the approach of previous investigators (with few exceptions[8,9]) has been to use approximations such as the linearized Poisson–Boltzmann equation[10] or Manning's limiting laws,[11] our studies comprise the first systematic analysis of the helix–coil transition of polyelectrolytes using the PBCM. In the PBCM, the full Poisson–Boltzmann equation is solved (i.e., no linearization approximation is introduced), and the concentrations of all species, including the polyion, are finite, which means that polyion–polyion interactions are considered implicitly. In our previous studies, we found both these features of the model to be essential for its providing a correct description of the electrostatic phenomena that were seen in the experimental results. Thus, in part 1, the effects of counterion valency and of mixed mono- and divalent counterions were studied, and some anomalous effects were found that (of the theories proposed to date) only can be accounted for by the PBCM. In part 2, the effect of added salt on the temperature-induced transition was studied, and both the shift in temperature and the change in sharpness of the transition with added salt could be explained by the PBCM, without necessitating any special assumptions such as a change in the cooperativity (which has been suggested previously in an attempt to explain the latter effect[12]).

Whereas parts 1 and 2 were both dealing with aqueous systems, we will in the present study investigate, experimentally and theoretically, the effects of replacing water by another solvent (primarily formamide). In the PBCM, the effect of the solvent enters only via its bulk dielectric constant, and it is of interest to see to what extent this single parameter may capture the changes in salt effects that (in real systems) accompany a change of the solvent. We have therefore repeated some of the experiments of part 1 using formamide as a solvent, and we will here investigate whether the similarities and the differences in the experimental results from the two different solvents, water and formamide, are in accord with the model predictions. Indeed, we will find this to be the case, and the success of the PBCM in accounting for salt effects also in another solvent lends further credibility to the model, demonstrating its usefulness and the soundness of its approximations. From a different aspect, the results and the analysis of the present study provide insights into the importance of general electrostatic contributions to the solvent effects seen in real polyelectrolyte systems.

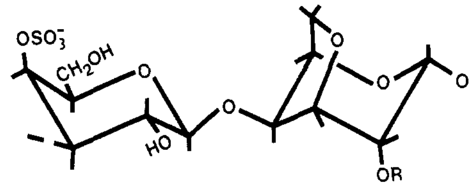

Figure 1. Repeating disaccharide structure of κ-carrageenan (R = H) and ι-carrageenan (R = SO_3^-).

As in our previous studies,[1,2] the substances studied in the present work are κ- and ι-carrageenan[3] (Figure 1), two moderately charged polysaccharides having one and two charges per repeating disaccharide unit, respectively. The carrageenans are well-known for their ability to form gels in water, where the network-generating mechanism is a coil-to-helix transition followed by the aggregation of helices.[3] The gel state is favored by high salt concentration and low temperature. As has been shown previously,[13] in formamide and dimethyl sulfoxide (DMSO) carrageenans still have the ability to undergo a helix–coil transition; however, they do not form gels and there is no indication of aggregate formation[13] (such as a thermal hysteresis in the conformational transition), as is sometimes found in water. To the best of our knowledge, the present work is the first systematic study of salt effects on the carrageenan conformational transition in nonaqueous solvents, the earlier studies[13,14] being

0024-9297/90/2223-2776$02.50/0

limited to κ-carrageenan in salt-free systems.

II. Experimental Section

Materials. Samples of ι-carrageenan (from *Eucheuma spinosa*, lot no. 124F-0605) and κ-carrageenan (from *Eucheuma cottonii*, lot no. 124F-0604) were obtained from Sigma Chemical Co. Segments of enhanced structural regularity were prepared as described by Bryce et al.[15] 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 weights. All carrageenan and salt samples used with formamide and DMSO as solvents were dried in a desiccator for at least 1 week to minimize any moisture content. The formamide was of pro analysii quality, and the DMSO was of spectral grade.

Methods. The helix–coil transition was monitored[3] by optical rotation measurements 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 through the jacketed cell. As in part 1, the helix formation onset temperature, T_o, 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 the compositions of systems that are characterized by a common T_o.

III. Theory and Model Parameters

The theoretical treatment of the helix–coil transition has been presented in detail in earlier papers.[1,2] The approach is to calculate the electrostatic contribution to the propagation parameter, s, in the Zimm–Bragg model for helix–coil transitions[4]

$$kT \ln s = m(\Delta\mu_{\mathrm{el}} + \Delta\mu_{\mathrm{non\text{-}el}}) = m(\Delta\mu_{\mathrm{el}} + \Delta H_{\mathrm{non\text{-}el}} - T\Delta S_{\mathrm{non\text{-}el}}) \quad (1)$$

Here, $\Delta\mu_{\mathrm{el}}$ and $\Delta\mu_{\mathrm{non\text{-}el}}$ represent the electrostatic and nonelectrostatic contributions to the helix propagation step, k and T have their usual meanings, m is the number of charged groups per repeating unit, and $\Delta H_{\mathrm{non\text{-}el}}$ and $\Delta S_{\mathrm{non\text{-}el}}$ are the nonelectrostatic enthalpic and entropic contributions (expressed per unit polyion charge) to the propagation parameter. Both $\Delta H_{\mathrm{non\text{-}el}}$ and $\Delta S_{\mathrm{non\text{-}el}}$ are assumed to be independent of temperature and electrolyte content. The electrostatic contribution, $\Delta\mu_{\mathrm{el}}$, to the helix propagation is given by the difference in the electrostatic chemical potential (expressed per polyion charge) between the helix and the coil conformations and is calculated within the PBCM. In this model, both polymer conformations are approximated as cylindrical rods of infinite length and uniform surface charge density, centered in cylindrical cells, the sizes of which depend on the overall composition of the system as well as on its helical content. The assumption of an infinitely long rod should be valid as long as the persistence length is longer than the Debye length. According to Slootmaekers et al.,[35] the persistence length of κ-carrageenan in the coil conformation extrapolated to infinite salt concentration is ca. 40–70 Å. The persistence length at any lower (finite) salt concentration is of course higher, and the above assumption should therefore be a very good approximation under the condition of this study.

Two parameters are needed to specify the dimensions of a model rod, i.e., its length per polyion charge, l, and its radius, a. In our earlier papers, with water as the solvent, the length per polyion charge was obtained from molecular models for the coil conformation and from X-ray diffraction data[16,17] on (hydrated) oriented fibers for the (double) helix conformation. The radius was then calculated from l and from the volume per unit charge of the model rod, which, for consistency reasons, was taken to be the same for both conformations. There exists no rigorous method by which "correct" model volumes may be obtained, and we simply used the corresponding partial molar volumes (in water) as determined experimentally[18,19] for carrageenans. (Within 1% these partial molar volumes are, in fact, independent of the carrageenan conformation.[19]) It could be argued that a contact radius, equaling the radius of a (solvated) ion, should be added to the bare polyion radii thus deduced and that, also for identical polyion conformations, the effective polyion radii could depend on the solvent. We have found, however (cf. below), that, within reasonable limits, the exact numerical values of the radii are not critical for the results obtained by the model. For this reason, and also in order to avoid adjustable parameters that would only obscure our results, we will in this work ignore the possible differences in effective polyion radii in different solvents, using the same model rod dimensions in formamide as we previously used for the aqueous case.[1,2] (We thus assume the ordered conformations of ι- and κ-carrageenan to be the same in formamide as in water; cf. below.) The carrageenan model parameters are listed in Table I.

Table I
Carrageenan Model Parameters

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

Table II
Temperatures (T_o ± 1/°C) for the Onset of Conformational Ordering of Carrageenans in Formamide in the Presence of Various 1:1 Electrolytes[a]

salt	$T_o(\iota)$	$T_o(\kappa)$
NaCl	52	63
LiCl	55	36
TMA-Cl	57	42

[a] The salt concentration was in all cases 0.5 M.

IV. Results and Discussion

Effects of Cation Specificity. As stated in the Introduction, the primary aim of this work is to study the effects of replacing water with another solvent (thereby changing the molecular interactions) on the carrageenan helix–coil transitions and to see to what extent the electrostatic part of these effects is captured by the PBCM, where the only solvent-dependent parameter is the dielectric constant [$\epsilon_r(\mathrm{H_2O}) = 78$ and $\epsilon_r(\mathrm{formamide})^{20} = 111$ at 25 °C]. Since the theoretical model only deals with unspecific electrostatic interactions, it is essential, however, to investigate whether ion-specific phenomena (which are well established in the case of aqueous κ-carrageenan systems[21] with monovalent counterions) occur in the particular solvent chosen. We have therefore studied the carrageenan conformational transitions in formamide in the presence of three different monovalent counterions ($\mathrm{Na^+}$, $\mathrm{Li^+}$, and $\mathrm{TMA^+}$ (tetramethylammonium)) and, for completeness, two different divalent ions ($\mathrm{Ca^{2+}}$ and $\mathrm{Ba^{2+}}$). In Tables II and III we present the helix formation onset temperatures, T_o, for the two carrageenans in formamide solutions of the chloride salts of these ions. The results clearly indicate that (as is the case in water[22]) there is no marked ion specificity for ι-carrageenan in formamide. The situation is less clear for κ-carrageenan

Table III
Temperatures (T_o ± 1/°C) for the Onset of Conformational Ordering of Carrageenans in Formamide in the Presence of Various 2:1 Electrolytes[a]

salt	$T_o(\iota)$	$T_o(\kappa)$
$CaCl_2$	52	38
$BaCl_2$	53	38

[a] 50 mM salt for ι-carrageenan and 140 mM salt for κ-carrageenan.

with monovalent counterions (there is no specificity with divalent ions), but the similarity of the results for the (chemically very different) Li^+ and TMA^+ ions suggests that the interactions of these ions with the polysaccharide is mainly of a Coulombic nature—all the more so as it is difficult to see how the TMA^+ ion could participate in any kind of specific binding. The Na^+ ion, on the other hand, is seen to weakly promote the formation of κ-carrageenan helices. This is different from the situation in water,[21] where Na^+, Li^+, and TMA^+ all give almost identical results, whereas K^+, Rb^+, and Cs^+ strongly promote the helix formation. To avoid effects from ion specificity, we have here chosen Li^+ as the monovalent counterion for κ-carrageenan in formamide, although we will compare the results obtained with earlier results[1] pertaining to the Na^+ form in water.

Together with the results from the earlier study[13] by Rochas and Rinaudo, our results indicate that the ions Na^+, K^+, and Rb^+ (Cs^+ seems not to have been tested) specifically favor the formation of κ-carrageenan helices in formamide. (Rochas and Rinaudo report helix formation for salt-free K^+ and Rb^+ κ-carrageenan samples[13] under conditions where our salt-free Na sample did not form helices. The helix-promoting ability in formamide of the former two ions is therefore much stronger than that of Na^+.) We also made some measurements on κ-carrageenan in DMSO, where we obtained a T_o value of 30 °C in the presence of 0.06 M NaCl, whereas concentrations as high as 1 M LiCl did not give any conformational ordering above the freezing point of the solvent (18 °C). Faced with these pronouncedly cation-specific results in DMSO, we did not pursue the investigation of the carrageenan conformational transitions also in this solvent, as was originally our intention. The fact that the ion specificity of κ-carrageenan involves different ions in different solvents seems not to have been observed previously. It is tempting to speculate that the mechanism of the ion specificity is a site binding, accompanied by partial desolvation of the counterion, as indicated by the chemical shifts observed in counterion NMR measurements.[23] The observed solvent dependence of the ion specificity would then reflect different solvation energies in different solvents.

Helix–Coil Transitions in Mixed Salts. In Figures 2 and 3 we present the experimental and theoretical conformational stability diagrams obtained in the present study of ι- and κ-carrageenan, in formamide, in the presence of mixed salts, together with analogous results for aqueous systems taken from our previous work.[1] Each of the four diagrams shows the concentrations of 1:1 and 2:1 electrolytes required to induce an onset of helix formation at some fixed temperature, T_o, which was chosen on grounds of experimental convenience. (Thus, T_o is different in all four diagrams. In this context, it should be noted that the electrostatic interactions in a given solvent are only marginally temperature dependent; cf. the near constancy of the product of $1/\epsilon_r$ and the Boltzmann factor in the Poisson–Boltzmann equation.) Each theoretical curve uses the (experimental) point with only monovalent counterions as an, arbitrarily chosen, reference point for which the value of $\Delta\mu_{el}$ was calculated. Since the helical content is constant and the propagation parameter is an equilibrium constant for the formation of helical units, it follows that the entire curve is characterized by a constant value of the propagation

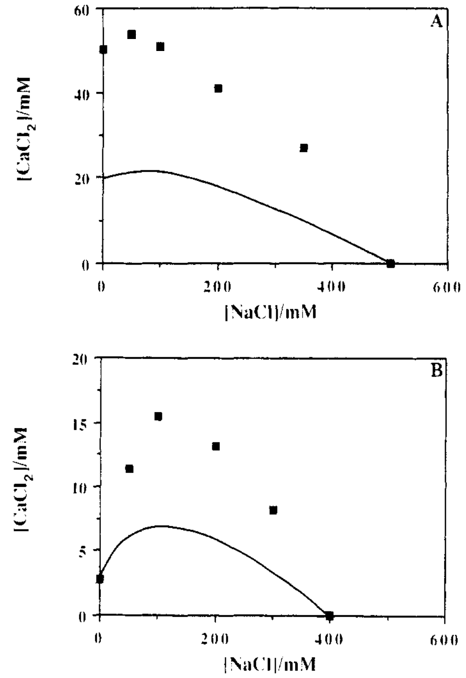

Figure 2. Conformational stability diagrams for ι-carrageenan (2.9 mM) in mixed electrolyte solutions in (A) formamide at 52 °C, ϵ_r = 98.2, and (B) water at 72 °C, ϵ_r = 63.3. The points indicate experimental salt concentrations required for the onset of helix formation at the respective temperatures, and the solid lines were calculated as described in the text.

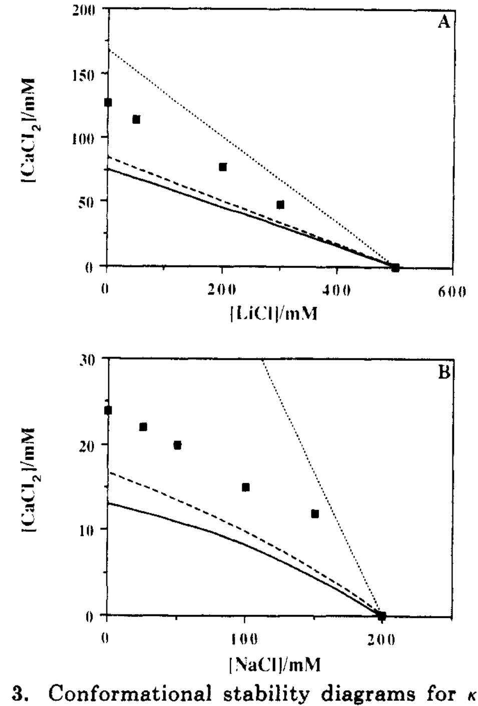

Figure 3. Conformational stability diagrams for κ-carrageenan (5 mM) in mixed electrolyte solutions in (A) formamide at 35 °C, ϵ_r = 104.9, and (B) water at 18 °C, ϵ_r = 81.0. The points indicate experimental salt concentrations required for the onset of helix formation at the respective temperatures. The theoretical curves refer to calculated results without (solid lines) and with (dashed lines) the inclusion of a 1-Å contact radius of the ions in the effective polyion radii. The dotted lines indicate conditions of constant ionic strength.

parameter or, equivalently (since temperature is constant), a constant value of $\Delta\mu_{el}$ (see part 1 for details). When analyzing the data in Figures 2 and 3, one should be aware that the nonelectrostatic contribution to the propagation parameter may of course be different in the two solvents; the value of $\Delta\mu_{non\text{-}el}$ is, however, not given by the present theory. In our treatment it is therefore not possible to predict, for instance, the shift in T_0 accompanying a change of solvent at a given salt concentration, since T_0 depends on both $\Delta\mu_{el}$ and $\Delta\mu_{non\text{-}el}$.

Figure 2 gives the stability diagrams for ι-carrageenan and, although the theoretical prediction underestimates the $CaCl_2$ concentrations by a factor of ca. 2.5 in formamide, it is evident that the order-of-magnitude increase in the concentrations of divalent ions following the replacement of water with formamide is well reproduced. Another striking feature of Figure 2 is the reduction of the (in water) pronounced maximum in the concentration of divalent ions; this maximum is almost absent in formamide. The same trend toward a disappearance of the nonmonotonic behavior is seen in the theoretical results; moreover, the [NaCl] coordinates of the respective maxima are well reproduced in both solvents. The theoretical basis for the occurrence of a maximum was given in part 1 in this series, and it was there found to be a general high-potential phenomenon. The maximum is therefore expected to be reduced in magnitude (and, eventually, to disappear) when the electrostatic potentials are reduced as is the case in a solvent with a higher dielectric constant.

Figure 3 shows the same kind of experiments as above, but for κ-carrageenan. (The additional dotted and dashed curves in these diagrams are explained below.) Here the curves (both experimental and calculated) are monotonic since the lower charge density of κ-carrageenan (compared to ι-carrageenan) gives rise to lower electrostatic potentials. The theoretically predicted concentrations of divalent ions are too low by a factor of ca. 2 in both solvents, but again the relative change in the concentrations of divalent ions following the change of solvent is well reproduced and so is the change in curvature of the diagrams: The stability diagrams obtained in water (Figure 3B) are distinctly curved whereas they are almost straight lines in formamide (Figure 3A).

The Helix Conformation. At this stage, it is appropriate to discuss our assumption that the ordered conformation of carrageenan is the same (i.e., a double helix) in formamide as in water. We may first conclude that there is nothing in the results presented above that would contradict this assumption. On the contrary, we find that as far as the comparisons between theory and experiments are concerned, all the features seen in aqueous solutions appear again in the case of formamide: The same systematic discrepancies are again apparent, and the stability diagrams change with the change of solvent in a manner totally in agreement with the predictions given by the model. Previously, however, Rochas and Rinaudo[13] have suggested (on the basis of calorimetric data and electrostatic considerations based on Manning's polyelectrolyte theory[11]) that the ordered conformation of κ-carrageenan does, in fact, differ in the two solvents, being a double helix in water and a single helix with half the linear charge density (i.e., one strand of the double helix) in formamide. We have tested the proposed single-helix conformation for κ-carrageenan in our model, and we found that these calculations actually give a better agreement with the experimental data of Figure 3A, yielding a straight line between the experimental points and the dotted line representing constant ionic strength. This result is, however, trivial, since in a low-potential regime any structure will give a result at or close to constant ionic strength. However, the overall conclusion of the results of Figure 3A must be that these are only mildly sensitive to the actual conformation of the κ-carrageenan helix.

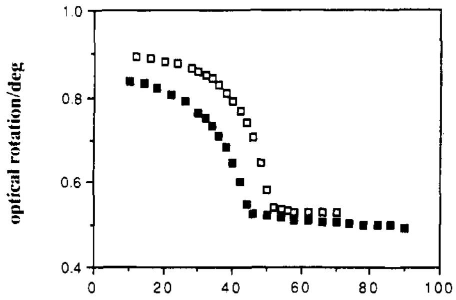

Figure 4. Conformational transition of κ-carrageenan (12 mM) as a function of temperature in the presence of 0.2 M TMA-iodide in formamide (■) and water (□).

As regards the proposed carrageenan single-helix structure, we do find this conformation highly unlikely, however, since such a conformation is expected to give rise to a totally noncooperative transition, as was pointed out in part 2. However, there is, of course, still a possibility that κ-carrageenan would assume some other helical structure, different from the aqueous double helix, in formamide. In fact, Rochas and Rinaudo[13] noted a broader transition (normally taken to indicate a decreased cooperativity) of κ-carrageenan in formamide and DMSO as compared to the aqueous case. However, the experiments reported in ref 13 were all performed in salt-free solutions, where (as was shown in part 2) electrostatic polyion–polyion interactions give rise to a transition broadening unrelated to the cooperativity of the transition. We have therefore compared (Figure 4) the conformational transition of κ-carrageenan in water and in formamide in the presence of 0.2 M TMA-iodide, where (at a polyion concentration of 12 mM disaccharide) electrostatic polyion–polyion interactions are of negligible importance. (Iodide salt was used since this prevents the formation of aggregates in water.[25]) Within experimental accuracy, the slopes of the two transitions shown in Figure 4 are identical, indicating[24] that the cooperativity of the transition is, at least, very similar in the two solvents. This, in turn, suggests that also the ordered conformations are closely similar. From the results in Figure 4 alone it is not possible to claim that the cooperativity is identical in the two solvents since the slope of a transition curve also depends on the transition enthalpy,[2,24] which, in general, is dependent on the medium. Unfortunately, there is an uncertainty in the literature regarding the value of the transition enthalpy in water, where values in the range 3–24 kJ/mol have been reported by different authors.[26-33] The only determination of the transition enthalpy in formamide[13] reported to data gives a value of 6 kJ/mol.

Model Deficiencies. As regards the source of the quantitative errors in the model there are, of course, several possibilities. One likely source is the use of a uniform surface charge density, which starts to become a questionable approximation when the Debye length is shorter than the linear charge separation of the polyion. The Debye length of a 0.5 M (1:1 electrolyte) salt solution is 5.1 Å in formamide and 4.3 Å in water, which should be compared with the charge separations (l) in Table I. To improve the situation it appears to be necessary to take the discrete nature of the polyion charges into account. This is difficult in general but could, in an extreme limit, be done by calculating the free energy by pairwise summation of spherical Debye–Hückel potentials over the polyion charges. The conditions for such an analysis are not quite satisfied in the present case, but the result would be the dotted straight lines representing constant ionic strength in the stability diagrams.

Another approximation of the PBCM is the neglect of pair correlations between counterions (the so-called mean-field approximation). This approximation is more serious the higher the charge density of the polyion becomes and is also more serious for divalent counterions than for monovalent counterions. From comparisons with Monte Carlo simulations[34] it has been found that for a polyelectrolyte in the presence of mixed counterions the PBCM underestimates the screening contribution from the divalent counterions. As was explained in part 1 the occurrence of a maximum in the $CaCl_2$ concentration in the stability diagrams (Figure 2) is due to the fact that the divalent ions can dominate the electrostatic potentials for a highly charged conformation. It therefore follows that an underestimate of the contribution from the divalent ions will account for the underestimate of the size of the maxima in the theoretical curves.

The quantitative errors that result from the above approximations cannot be assessed without considerable effort—all the more so as the desired quantities are *differences* in the chemical potentials of the two conformational states. One easily tested source of error lies, however, in the values chosen to represent the model polyion radii (cf. Table I). The sensitivity of the results to the parameter a is indicated by the dashed lines in Figure 3. These lines correspond to calculations where, compared to the values given in Table I, 1 Å has been added to radii of both the helix and the coil conformations (the volume per polyion charge is therefore different for the two conformations in this case). Physically, this increase of all radii by a constant amount would correspond to the introduction of a finite (and constant) contact radius of the ions. However, judging from the results in Figure 3, changes in the (effective) polyion radii by amounts of the order of 1 Å will only marginally affect the calculated results. We may therefore conclude that the polyion radius is not a critical parameter.

V. Summary

In this work, we have extended our investigation of electrostatic effects on the helix–coil transition of ionic polymers to the case of nonaqueous solvents. As in part 1, we find that the PBCM captures the essential qualitative features of real systems in the presence of mixed mono- and divalent counterions, and a semiquantitative agreement with experimental data on the helix–coil transitions of κ- and ι-carrageenan is obtained. The effects of replacing water by formamide as the solvent are correctly predicted by only taking the dielectric constants into account, assuming the same ordered conformation in both solvents. The present study has further verified the prediction, made in part 1, that the nonmonotonic conformational stability diagrams found in mixed-salt solutions under high-potential conditions should approach a monotonic behavior (with a constant ionic strength as a limiting case) when the electrostatic potentials are reduced. The effect of adding in the calculations a contact radius to the polyion radius as an attempt to take the counterion size into account has been tested and was found to only marginally affect the results. As in water, an ion specificity of the transition of κ-carrageenan (but not ι-carrageenan) was found also in nonaqueous solvents; however, the ion specificity involves different ions in different solvents.

Acknowledgment. A grant from STU, the Swedish National Board for Technical Development, is gratefully acknowledged.

References and Notes

(1) Nilsson, S.; Piculell, L.; Jönsson, B. *Macromolecules* **1989**, *22*, 2367.
(2) Nilsson, S.; Piculell, L. *Macromolecules* **1989**, *22*, 3011.
(3) 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.
(4) Zimm, B. H.; Bragg, J. K. *J. Chem. Phys.* **1959**, *31*, 526.
(5) Fuoss, R. M.; Katchalsky, A.; Lifson, S. *Proc. Natl. Acad. Sci. U.S.A.* **1951**, *37*, 579.
(6) Alfrey, T.; Berg, P. W.; Morawetz, H. *J. Polym. Sci.* **1951**, *7*, 543.
(7) Gunnarson, G.; Jönsson, B.; Wennerström, H. *J. Phys. Chem.* **1980**, *84*, 3114.
(8) Nagasawa, M.; Muroga, Y. *Biopolymers* **1972**, *11*, 461.
(9) Jamnik, A.; Bratko, D. *Vestn. Slov. Kem. Drus.* **1987**, *34*, 15.
(10) Peller, L. *J. Phys. Chem.* **1959**, *63*, 1199.
(11) Manning, G. S. *Q. Rev. Biophys.* **1978**, *11*, 179.
(12) Dove, W. F.; Davidson, N. *J. Mol. Biol.* **1962**, *5*, 467.
(13) Rochas, C.; Rinaudo, M. *Carbohydr. Res.* **1982**, *105*, 227.
(14) Rochas, C.; Rinaudo, M.; Vincendon, M. *Biopolymers* **1980**, *19*, 2165.
(15) Bryce, T. A.; Clark, A. H.; Rees, D. A.; Reid, D. S. *Eur. J. Biochem.* **1982**, *122*, 63.
(16) Anderson, N. S.; Campbell, J. W.; Harding, M. M.; Rees, D. A.; Samuel, J. W. B. *J. Mol. Biol.* **1969**, *45*, 85.
(17) Arnott, S.; Scott, W. E.; Rees, D. A.; McNab, C. G. A. *J. Mol. Biol.* **1974**, *90*, 253.
(18) Gekko, K.; Mugishima, H.; Koga, S. *Int. J. Biol. Macromol.* **1985**, *7*, 57.
(19) Gekko, K.; Kasuya, K. *Int. J. Biol. Macromol.* **1985**, *7*, 299.
(20) Dunn, L. A.; Stokes, R. H. *Trans. Faraday Soc.* **1969**, *65*, 2906.
(21) Rochas, C.; Rinaudo, M. *Biopolymers* **1980**, *19*, 1675.
(22) Piculell, L.; Håkansson, C.; Nilsson, S. *Int. J. Biol. Macromol.* **1987**, *9*, 297.
(23) Grasdalen, H.; Smidsrød, O. *Macromolecules* **1981**, *14*, 229.
(24) Poland, D.; Scheraga, H. A. *Theory of Helix-Coil Transitions in Biopolymers*; Academic Press: New York, 1970.
(25) Grasdalen, H.; Smidsrød, O. *Macromolecules* **1981**, *14*, 1845.
(26) Rochas, C.; Mazet, J. *Biopolymers* **1984**, *23*, 2825.
(27) Cesaro, A.; Delben, F.; Paoletti, S.; Scagnolari, F. *Termochim. Acta* **1985**, *85*, 465.
(28) Paoletti, S.; Delben, F.; Cesaro, A.; Grasdalen, H. *Macromolecules* **1985**, *18*, 1834.
(29) Rochas, C. *J. Therm. Anal.* **1985**, *30*, 1375.
(30) Norton, I. T.; Goodall, D. M.; Morris, E. R.; Rees, D. A. *J. Chem. Soc., Faraday Trans.* **1983**, *79*, 2475.
(31) Snoeren, T. H. M.; Payens, T. A. J. *Biochim. Biophys. Acta* **1976**, *437*, 264.
(32) Norton, I. T.; Morris, E. R.; Rees, D. A. Carbohydr. *Res.* **1984**, *134*, 89.
(33) Morris, E. R.; Rees, D. A.; Norton, I. T.; Goodall, D. M. *Carbohydr. Res.* **1980**, *80*, 317.
(34) Murthy, C. S.; Russel, J. B.; Rossky, P. J. *J. Phys. Chem.* **1985**, *89*, 701.
(35) Slootmaekers, D.; De Jonghe, C.; Reynaers, H.; Varkevisser, F. A.; Bloys van Treslong, L. J. *Int. J. Biol. Macromol.* **1988**, *10*, 160.

Registry No. DMSO, 67-68-5; H_2O, 7732-18-5; $HCONH_2$, 75-12-7; NaCl, 7647-14-5; LiCl, 7447-41-8; $Me_4N^+Cl^-$, 75-57-0; $CaCl_2$, 10043-52-4; $BaCl_2$, 10361-37-2; ι-carrageenan, 9062-07-1; κ-carrageenan, 11114-20-8.