

Conformation-Dependent Interactions Between Ionic Polysaccharides and Counterions in Dilute Aqueous Solution Potentiometric, Calorimetric, and Optical Activity Studies

V. Crescenzi, M. Dentini, G. Paradossi and R. Rizzo

Istituto di Chimica Fisica, Università di Roma, Roma, Italy

Summary

The interaction of Cu^{2+} ions with dextran sulfate and with i-carrageenan, respectively, in dilute aqueous solution has been studied by means of potentiometric, optical rotation, and micro-calorimetric measurements.

Distinct anomalies are found in the physico-chemical behaviour of i-carrageenan as a consequence of Cu^{2+} binding, which are ascribed to a conformational transition of the polysaccharide chains.

Introduction

The thermally induced conformational transitions of intact i-carrageenan as well as of segmented i-carrageenan in aqueous salt solution have already been investigated and the conformational stability of the ionic polysaccharides studied as a function of added NaCl or CaCl_2 concentrations, (REES, WELSH 1977; KARIMAN 1978).

Increasing counterions activity steadily stabilizes an ordered state of i-carrageenan chains, as normally evidenced by an increase in the optical activity of the latter (at around room temperature) and by a shift toward higher temperatures of the sigmoidal optical activity-temperature profiles associated with the order-disorder cooperative transition.

Linear i-carrageenans may therefore be considered to be in a randomly coiled state in dilute aqueous solution (at around 25°) allowance being made for the characteristic stiffness of the polysaccharide chains, while upon addition of less than equivalent amounts of divalent counterions and consequent extensive binding

of the latters, they would assume a helical conformation (REES, WELSH 1977).

We have studied the interaction between i-carrageenans (Na^+ salt) and Cu^{2+} ions in dilute aqueous solution by means of potentiometric, calorimetric, and optical activity measurements. For comparison purposes similar measurements have also been carried out using dextran sulfate (Na^+ salt) a highly charged, branched synthetic ionic polysaccharide.

The aim of our study has been primarily that of characterizing from the energetic stand point the salt-induced coil \rightarrow helix isothermal (28°) conformational change of i-carrageenans.

Potentiometric measurements

The activity coefficient of Na^+ counterions in i-carrageenan and dextran sulfate aqueous solutions, respectively, has been evaluated in function of polymer concentration as well as in function of added $\text{Cu}(\text{NO}_3)_2$ concentration (at fixed polymer concentration) by means of potentiometric measurements using a Na-glass electrode (see Experimental Part).

The results are reported in Fig.1. From these data it clearly appears that:

- a) the Na^+ activity coefficient (γ_{Na^+}) is quite low, in particular in dextran sulfate solutions and, moreover, is nearly independent of polyelectrolyte concentration at least in the limited range of dilutions investigated. Our γ_{Na^+} data are in good agreement with values reported by others in the literature, (KARIMAN 1978).
- b) addition of Cu^{2+} ions initially promotes the release of practically equivalent amounts of Na^+ ions from the macroions domains, for both i-carrageenan and dextran sulfate. In other words, Cu^{2+} ions are nearly quantitatively bound by the polysaccharidic chains under the experimental conditions employed. On further increasing the stoichiometric Cu^{2+} concentration, the activity coefficient of Na^+ ions is seen to reach a constant maximum value, of about 0.8 in the case of i-carrageenan, and near to unity in the case of dextran sulfate.

In all cases, however, the γ_{Na^+} vs $[\text{Cu}^{2+}]/N$ plots do not exhibit any discontinuity or anomaly.

Optical activity measurements

The results of the optical activity measurements are reported in Fig. 2. From these data it is evident that in the case of i-carrageenan as opposed to the case of dextran sulfate the $[\alpha]_{303}$ values vary in a discontinuous manner with increasing Cu^{2+} concentration. It appears logical to assume that this finding may be traced back to a " Cu^{2+} binding"-induced conformational transition of i-carrageenan, in analogy with evidences reported by others, (KARIMAN 1978), and collected in this laboratory for the system Ca^{2+} -i-carrageenan.

Calorimetric measurements

The results of the calorimetric measurements are reported in Fig. 3 and 4. In Fig. 3 the enthalpy of dilution of segmented i-carrageenan and of dextran sulfate in water at 28° is given as a function of $\lg N$, where N is the polyelectrolyte concentration in equiv/l.

These data have been useful also for correcting the heat exchanges measured on mixing polysaccharide and $\text{Cu}(\text{NO}_3)_2$ solutions.

The results of the latter measurements, expressed as calories per equivalent of polymer, are given in Fig. 4 as a function of the $[\text{Cu}^{2+}]/N$ ratio (at 28°).

Interesting enough, it clearly appears that while in the case of dextran sulfate the enthalpy of Cu^{2+} binding is always positive (the limiting differential enthalpy of binding, ΔH_B , in calories per mole of bound Cu^{2+} ions, as evaluated from the initial slopes of the plot of Fig. 4 results 1 Kcal/mol for NaDS and 0,7 Kcal/mol for i-carrageenan) in the case of i-carrageenan it changes sign for $[\text{Cu}^{2+}]/N \sim 0,7$. This peculiar effect has been found using both segmented and intact i-carrageenan samples.

Discussion

With the exception of the Na^+ activity coefficient data, our results clearly point out that the addition of Cu^{2+} ($\text{Cu}(\text{NO}_3)_2$) ions to a dilute solution of either segmented or intact i-carrageenan

in water leads to a marked change in physico-chemical properties of the polysaccharide.

For our experimental conditions, this change takes place in a rather abrupt manner for a $[\text{Cu}^{2+}]/N$ ratio around 0.7. Comparing the results of the optical activity measurements (Fig. 2) and of the microcalorimetric ones (Fig. 4) and taking into account spectroscopic and viscosity data from previous studies, (KARIMAN 1978), one may conclude that the above said results are evidence of a conformational transition of *i*-carrageenan brought about by Cu^{2+} - binding.

Assuming that, were not for the energy change associated with the conformational transition the enthalpy of Cu^{2+} binding by *i*-carrageenan should be always positive, as it has been found in the case of dextrane sulfate the enthalpy of transition would be of the order of -1 Kcal perequivalent of polyelectrolyte, as a rough estimate (see Fig. 4).

The potentiometric data (Fig. 1) would not be of help in probing the conformational transition of *i*-carrageenan because of the relatively limited accuracy of the Na^+ data and/or because the transition by itself is of little consequence for what concerns the average activity coefficient of Na^+ counterions.

Regarding the nature of the conformational transition, i.e. whether it involves single helix or double helix formation, no definitive conclusion can be drawn from our present data. It may be supposed, however, from the calorimetric data (Fig. 4) that the nature of the transition is likely to be the same for segmented and for intact *i*-carrageenan chains.

Finally, the heat of dilution data (Fig. 3) deserve a few comments. The theoretical treatment of polyelectrolyte solutions due to MANNING (1978), predicts that the enthalpy of dilution of charged linear chains with monovalent counterions in water should be given by (ISE, OKUBO, 1978):

$$(\Delta H \text{ dil})_{N \rightarrow N^*} = \frac{RT}{2\xi} \left(1 + \frac{T}{D} \frac{dD}{dT} \right) \ln \frac{N}{N^*} \quad (1)$$

in which N^* is a reference polyelectrolyte concentration where

dilution was carried out from N , D is the dielectric constant, and where the important parameter ξ , $\xi = e^2 / DKT b$, (2) depends essentially on the distance b between neighboring charges on the macroion (e is the electronic charge and K is the Boltzmann's constant).

Evaluating ξ as 1, 2 and 2,8 for our samples of segmented i-carrageenan and of dextran sulfate, respectively (see Experimental part), and assuming, as is quite logical, that the factor $(1 + \frac{T}{D} \frac{dD}{dT})$ is the same for dilute aqueous solutions of the two polymers, one concludes that the slopes of the ΔH_{dil} vs. $\lg N$ curves for i-carrageenan and dextran sulfate should be in the ratio of 2,8/1,2 = 2.4 according to equations (1) and (2).

It is easily verified that this is in fair agreement with our results reported in Fig. 3 (initial slopes ratio: 2,1). More extensive calorimetric measurements are currently under way using intact i-carrageenan and K-carrageenan: for the latter, the least charged among carrageenans, one should find in fact the largest ΔH_{dil} on going from any given N to N^* (eq. (1)), at high dilutions. (if $\xi > 1$)

Experimental

The segmented i-carrageenan sample was a kind gift of Dr. S. REID, Unilever Research Laboratory (England).

The intact i-carrageenan sample was a SIGMA-4 (USA) product, purified and characterized in the CERMAV laboratory of the University of Grenoble (France). Dextran sulfate of $\bar{M} 5.10^5$ and 4.10^4 were PHARMACIA (Sweden) samples, which have been purified by dialysis.

Elemental analysis of the above said polysaccharides (Na salts) and potentiometric titrations carried out after conversion to the free acids by ion exchange have yielded the following equivalent-weight data:

- 1) i-carrageenan segments, 270; 2) intact i-carrageenan, 270;
- 3) dextran sulfate, 219, taking of course into account the different water contents of the samples.

These values correspond to a degree of substitution of 2.0

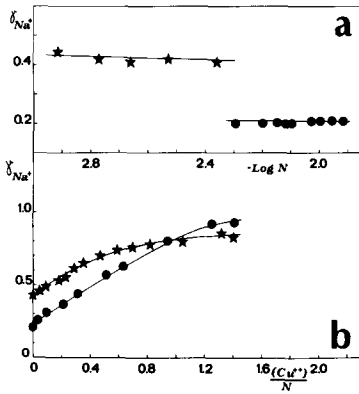


Fig. 1 a) Na⁺ counterions activity coefficient as a function of polymer concentration (N, equiv/l) for:

★ : dextran sulfate; $\bar{M}=4,10^4$
 ● : i-carrageenan (intact).

b) dependence of the Na⁺ activity coefficient at 25° in sodium dextran sulfate (★) and i-carrageenan (●) aqueous solutions, on the stoichiometric, equivalent concentration ratio $[Cu^{2+}]/N$. Polyelectrolyte concentration: $N=4,8 \times 10^{-3}$ equiv/l.

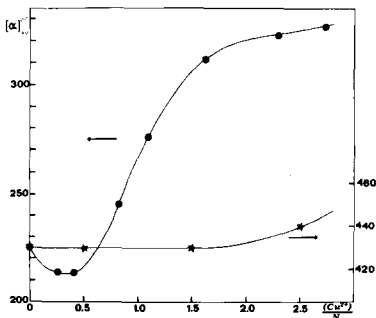


Fig. 2 - Dependence of the optical activity at 303 nm and 25° of i-carrageenan (intact, ●), and of dextran sulfate ($\bar{M} = 4.10^4$, ★), on the stoichiometric, equivalent concentration ratio, $[Cu^{2+}]/N$. Polyelectrolyte concentration: $N = 1.8 \times 10^{-3}$ equiv/l.

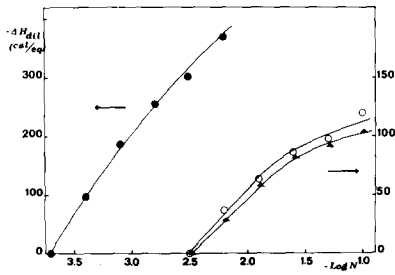


Fig. 3 - Enthalpy of dilution of sulfated polysaccharides (sodium salts) in water at 28° (see text)
 ▲ : dextran sulfate; $M=4 \times 10^4$
 ○ : dextran sulfate; $M=5 \times 10^5$
 ● : i-carrageenan (segments)

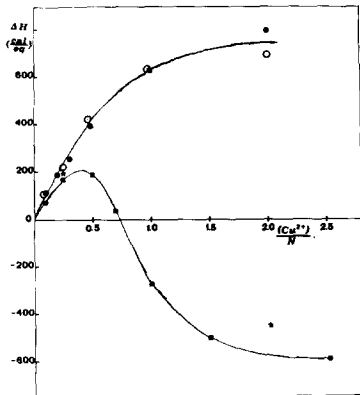


Fig. 4 - Enthalpy of interaction between sulfated polysaccharides and Cu^{2+} ions in water at 28°
 ● : dextran sulfate; $\bar{M}=4.10^4$
 ○ : dextran sulfate; $\bar{M}=5.10^5$
 ■ : i-carrageenan (segments)
 ★ : i-carrageenan (intact)
 Polyelectrolyte concentration:
 $N = 2 \times 10^{-3}$ equiv/l.

for dextran sulfate (number of sulfate groups per glucose residue) and of 1,7 for i-carrageenan segments (average number of sulfate groups per two sugar residues in the chain),

Copper nitrate solutions were prepared using a pure $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ C. ERBA (Italy) sample: the titre of the solutions was controlled by titration of HNO_3 liberated upon passage through an ion-exchange column.

All solutions were prepared using deionized, bidistilled water.

Optical activity measurements were carried out using a Perkin-Elmer 141M spectropolarimeter. The temperature of the cells (28°) was controlled by circulating water through an ultrathermostat.

Calorimetric measurements were performed using a LKB 10070-batch microcalorimeter at 28°C following a procedure similar to that already described elsewhere, (PAOLETTI, DELBEN, CRESCENZI, 1976).

Potentiometric measurements for the evaluation of Na^+ activity coefficients were carried out using a Orion 801 Analyser with a Orion Na-electrode in conjunction with a reference calomel electrode and a water jacketed titration cell.

Acknowledgements: This work has been carried out with financial support of the Italian CNR.

References

- 1) S.PAOLETTI, F.DELBEN and V.CRESCENZI: *J.Phys.Chem.*, 80, 2564 (1976)
- 2) N.ISE, T.OKUBO: *Macromolecules*, 11, 439 (1978)
- 3) A.KARIMAN: Thèse de doctorat de 3e cycle: "Caracterisation et propriété en solution des K, i, λ -carraghénanes", University of Grenoble (1978)
- 4) G.S.MANNING: *J.Chem.Phys.*, 51, 924 (1969)
- 5) D.A.REES, E.J.WELSH: *Ang.Chem.Int.Ed.Engl.*, 16, 214 (1977)

Received August 3, 1979