CHEMISTRY LETTERS, pp. 763-766, 1994.

An Investigation of the Ionic Polarization of Carrageenans with Different Charge Densities

Kazuyoshi UEDA, Shigenobu SATO, Hiroshi OCHIAI,
Akira IMAMURA, and Kiwamu YAMAOKA
Department of Chemistry, Faculty of Science, Hiroshima University,
Higashi-Hiroshima, Hiroshima 724

Counterion polarization of κ-, ι- and λ-carrageenans, which have different charge densities, in aqueous solution was investigated through the electric birefringence method. The data analysis by the Manning's counterion condensation theory suggests that the loosely bound counterions mainly contribute to the ionic polarization.

Polyelectrolyte molecule in aqueous solution can be oriented by the external electric field and shows a large electric birefringence.[1,2] The main origin of its orientation is ascribed to the torque exerted on an ionic polarization moment by the electric field. This polarization occurs in the displacement of the counterions surrounding the polyion. As for the counterions around the polyion, some of them are strongly attracted and bound to the vicinity of the polyion due to the high electrostatic potential of the polyion. Manning shows in his counterion condensation theory that those bound counterions are classified into two types (loosely and condensed) by the charge density parameter of the polyion ξ.[3] ($\xi = e^2/\varepsilon kTb$, b: the charge distance of the polyion) If $\xi<1$, only the one type of bound counterion (loosely bound) exists. On the contrary, if $\xi>1$, some of them are tightly bound to the polyion (condensed). However, whether the counterions responsible for the ionic polarization are 'tightly bound' counterions to the vicinity of the polyion[4,5] and/or 'loosely bound' counterions,[6-8] has not been clarified yet, and is still under controversial. One of the reason is that the previous experiments are all carried out by using the samples of synthetic polyelectrolytes and polynucleotides with $\xi>1$.

Under the above circumstance, to specify the ionic polarization mechanism, the use of carrageenans is very suitable, which are stiff acidic polysaccharides and have different number of ionizable groups per disaccharide unit. That is , the charge density parameter for κ-carrageenan is $\xi<1$, and ι- and λ-carrageenans are $\xi>1$, respectively. In the previous electric birefringence study of κ-carrageenan,[9] we demonstrated that the loosely bound counterions alone can induce a large ionic polarization. But, the details of its polarization magnitude could not be analyzed. In this study, the concentration dependence of the electric birefringence of κ-, ι- and λ-types of carrageenans was determined and their polarization magnitudes were compared each other. The results again suggest the importance of the loosely bound counterions to the ionic polarization.

κ- and ι-Carrageenans were gifted from Mitsubishi Rayon, Co. Ltd. (Tokyo) and λ-carrageenan was purchased from Sigma Chemical Co. Ltd. Each of them was sonicated separately in aqueous solution and fractionated by use of step-wise precipitation with 2-propanol.[9] The sample fractions with similar degrees of polymerization were selected from the fractions for each κ-, ι- and λ-carrageenan, respectively. The degree of polymerization (D.P.) was determined by GPC-LALLS (Tosoh Co. Ltd., Tokyo). The sulphate contents per disaccharide unit (D.S.) were determined by the potentiometric titration. These data are shown in Table 1. The values of ξ were calculated from the data of D.S.[3] All the samples were converted to K^+-salt by the procedure described in the literature.[10] The measurement and the apparatus of the electric birefringence were the same in the previous papers.[9,11]

The electric field strength dependence of the electric birefringence (Δn) of κ-, ι- and λ-carrageenans in aqueous solution was measured at various polymer

Table 1. Characteristic molecular parameters of various carrageenans

	κ-type	ι-type	λ-type
D.P.	156	177	179
D.S.	0.9	1.8	2.4
ξ	0.64	1.26	1.68

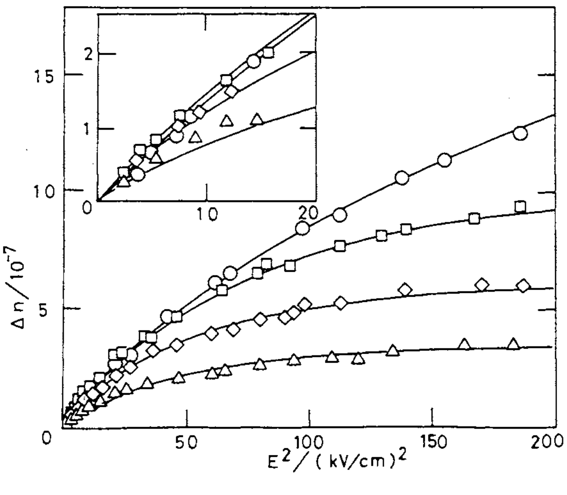

Fig. 1. Dependence of the electric birefringence on E^2 of ι-carrageenan. Concentrations are 0.46 mM (○), 0.23 mM (□), 0.12 mM (◇), and 0.06 mM (△), respectively. Inset is the low field region. The solid lines are theoretical (see text).

concentrations. All samples exhibit the similar dependence of Δn on the square of the electric field strength, E^2. Figure 1 shows the typical Δn *vs.* E^2 plot for ι-carrageenan. At lower field strength, the value of Δn is proportional to E^2, that is, the Kerr law holds (see inset). But, they tend to saturate at higher field strength. In order to determine the ionic polarizability ($\Delta\alpha$) defined in the previous paper,$^{9)}$ the observed field strength dependence of Δn was fitted to the theoretical curve of the orientation function (SUSID) proposed by Yamaoka and Fukudome.$^{12,13)}$ The best fitted theoretical curves for ι-carrageenan are shown by the solid lines in Fig. 1 . The values of $\Delta\alpha$ thus obtained from these curve fittings are plotted as a functions of polymer concentration in Fig. 2. The concentration dependence of $\Delta\alpha$ for all samples is quite similar in each other, in spite of their different charge densities. The values of $\Delta\alpha_0$, extrapolated to zero concentration, are given in Table 2 and they are much the same in each other.

By using the Manning's limiting law,$^{3)}$ the number of loosely bound and tightly bound (condensed) ions can theoretically be calculated from the values of ξ and the number of charged sites on the polyion. Those values for κ-, ι- and λ-carrageenans are shown in Table 2. If we suppose that only the tightly bound counterions can contribute to the polarization, it is expected that the polarizability of λ-type is 2.5 times larger than that of ι-type and κ-type is

Table 2. The ionic polarizability at infinite dilution and the number of charged sites of the carrageenans, and free, loosely and tightly bound counterions calculated from Manning's theory

	κ-type	ι-type	λ-type
$\Delta\alpha_0/10^{-32}$ F·m^2	8.8	10.2	8.9
Charged Sites	140	319	430
Free.	102	153	155
Loosely Bound	38	100	101
Tightly Bound	0	66	174

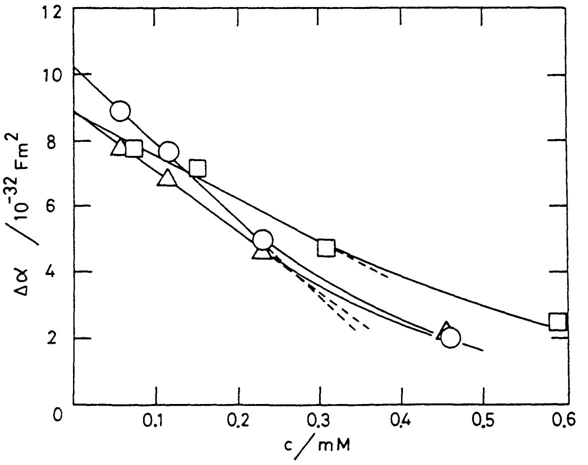

Fig. 2. Concentration dependence of the ionic polarizability. Symbols are for the κ-carrageenan (□), ι-carrageenan (○), λ-carrageenan (△), respectively.

zero-polarizability, taking into consideration the proportionality of ionic polarization to the number of polarizable counterions.$^{6)}$ But, the experimental $\Delta\alpha_0$ for κ-type is not zero and those are much the same for all types of carrageenans. These do not support the above supposition. On the other hand, if only the loosely bound counterions can contribute to the polarizability, the experimental results can be qualitatively interpreted. Especially, ι- and λ-types having the same number of loosely bound counterions have nearly the same polarizability. However, the relatively large $\Delta\alpha_0$ value of κ-type cannot be interpreted satisfactorily, since the calculated number of loosely bound counterion for κ-type is about one third of those for ι and λ. This may imply that some other binding mechanism contributing to the ionic polarization must be considered. Probably, the flexibility and the conformation of these polysaccharides may affect the binding state of counterions. While we cannot explain unambiguously the whole mechanism from the present data, the above lines of evidences strongly suggest that the loosely bound counterions can be considered as the main factor for the ionic polarization. Further studies will be done in the near future.

We thank Daicel Chemical Co., Ltd. for financial support.

References

1) E. Fredericq and C. Houssier, "Electric Dichroism and Electric Birefringence,"Clarendon Press, Oxford (1973).
2) "Molecular Electro-Optics, Part 2," ed by C. T. O'Konski, Marcel Dekker, New York (1978).
3) G. S. Manning, *J. Chem. Phys.*, **51**, 924 (1969).
4) G. S. Manning, *Biophys. Chem.*, **9**, 65 (1978).
5) E. Charney, K. Yamaoka, and G. S. Manning, *Biophys. Chem.*, **11**, 167 (1980).
6) M. Mandel, *Mol. Phys.*, **4**, 489 (1961).
7) C. Hornick and G. Weill, *Biopolymers*, **10**, 2345 (1971).
8) D. C. Rau and E. Charney, *Biophys. Chem.*, **14**, 1 (1981).
9) K. Ueda, H. Ochiai, T. Itaya, and K. Yamaoka, *Polymer*, **33**, 429 (1992).
10) K. Gekko, H. Mugishima, and S. Koga, *Int. J. Biol. Macromol.*, **9**, 146 (1987).
11) The apparatus used is constructed in our laboratory. The details of the apparatus, see; K. Yamaoka and Y. Hino, *Bull. Chem. Soc. Jpn.*, **62**, 251 (1989).
12) K. Yamaoka and K. Fukudome, *J. Phys. Chem.*, **92**, 4994 (1988).
13) K. Yamaoka and K. Fukudome, *J. Phys. Chem.*, **94**, 6896 (1990).

(Received January 17, 1994)