ι-Carrageenan Is Excluded from the Chiral Nematic Phase of κ-Carrageenan

Lennart Piculell,*,† Tobias Sparrman,‡ Mohamed Ramzi,† Johan Borgström,† and Per-Ola Quist‡

Physical Chemistry 1, Center of Chemistry and Chemical Engineering, Lund University, Box 124, S-221 00 Lund, Sweden, and Physical Chemistry, Umeå University, S-901 87 Umeå, Sweden

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Introduction. Carrageenans are linear, sulfated polygalactans from marine red algae. κ-Carrageenan (KC) and ι-carrageenan (IC) are the two most studied helix-forming varieties, well-known for their gelling properties.[1] They differ chemically only by the number of sulfate ester groups (one or two, respectively) in the repeating disaccharide unit (Figure 1). Both form helices upon cooling under appropriate salt conditions, and the helix formation is a necessary—but not sufficient—condition for gelation. It is notable that KC and IC do not form mixed double helices: In a mixed solution, each variety forms helices on its own at its expected transition temperature.[2−6]

As we have recently demonstrated, KC may also form nematic solutions: A sufficiently concentrated solution (above ca. 5%) of ultrasonically degraded KC in certain iodide salt solutions phase separates into two liquid phases, one chiral nematic and one isotropic.[7,8] In the course of analyzing this phase behavior by ^{13}C NMR, we unexpectedly discovered that IC, which is normally present as a contamination in KC,[3−6,9,10] is strongly excluded from the nematic phase. Here we report on this finding.

Experimental Section. Materials. KC (from *Eucheuma cottonii*) was a kind gift from SBI, France. The sample was precipitated in 2-propanol (to remove excess salt), ion-exchanged to the sodium form at 90 °C, and depolymerized for 2 h by ultrasonication to yield a weight-average molecular weight of approximately 7×10^4 g/mol. Details on the sample preparation have been given previously.[7,11]

Sample Preparation. Freeze-dried sodium KC was mixed with 0.1 M aqueous alkali metal iodide solutions at the desired composition. Concentrations of sodium KC are given as weight percent (wt %) throughout. The mixtures were heated at 85 °C for at least 30 min with stirring. Clear, monophasic solutions (the "mother solutions") of KC in the random coil configuration were thus obtained. Each mother solution was then left at room temperature overnight, whereupon a phase separation (induced by the coil-to-helix transition of KC) occurred. Centrifugation at 25 °C for at least 72 h at 4500 g yielded two clear fluid phases separated by a sharp meniscus: One isotropic top phase and one birefringent bottom phase. In addition, a small volume of a turbid precipitate of unknown composition collected at the bottom. The precipitate was discarded, and the individual liquid phases (isotropic and nematic) were taken out and analyzed separately.

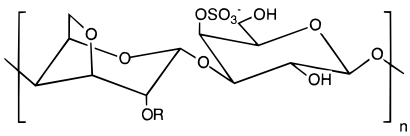

Figure 1. Repeating disaccharide units of KC (R = H) and IC (R = SO_3^-).

NMR Measurements. ^{13}C NMR spectra with WALTZ-16 proton decoupling were run in both the Lund and the Umeå laboratories, using the following equipment and parameters. Lund: 2048 FIDs with a spectral width of 15 kHz were acquired with a recycle delay of 0.6 s on a Varian 600 Unity Plus and Unity Inova spectrometer at a resonance frequency of 150.85 MHz. Umeå: 3000 FIDs with a spectral width of 20 kHz were acquired with a recycle delay of 2.5 s on a Chemagnetics Infinity-400 spectrometer operating at 100.713 MHz.

Chemical Analysis. Elemental analysis was performed by Mikro Kemi AB, Uppsala, Sweden. Carbon and sulfur were determined by catalytic oxidation at elevated temperature (1800 °C) in a controlled He/O_2 atmosphere, followed by gas chromatography to separate the different oxides (the so-called "Carlo Erba" method). Water was determined gravimetrically, and Cs and Na were determined by atomic flame emission spectroscopy.

Results. Figure 2 compares the ^{13}C NMR spectrum of a 6% KC mother solution with the spectra from the corresponding nematic and isotropic phases that separated out at 25 °C. All spectra were recorded under identical conditions at 80 °C, where the carrageenan was completely converted to the random coil configuration. The IC contamination gives rise to some characteristic peaks[12] that are well resolved from the KC spectrum (Figure 2). These peaks clearly show that IC was present in the mother solution, enriched in the isotropic phase, and virtually absent from the nematic phase. The origin of the peaks was further confirmed by additional NMR experiments on pure IC and on KC samples to which some pure IC had been added (not shown). The latter samples were analyzed before and after phase separation, and the added IC behaved just like the IC contamination originally present: The IC peaks grew in size, but IC was strongly excluded from the nematic phase. On increasing the total concentration of KC (without added IC), the volume of the isotropic phase decreased, and the proportion of the IC impurity in this phase then increased, as expected.

Figure 3 shows the temperature dependence of the ^{13}C spectrum from a separated isotropic phase of a 6% (initial concentration) sample. In accordance with previous findings,[13] the intensity of the high-resolution ^{13}C spectrum decreased on cooling as a result of the successive transition of carrageenan to the rigid, helical state. Note that this occurred also for the IC contamination in the investigated sample; the A1 signal in Figure 3 has vanished completely at 25 °C. Thus, the IC molecules were in the helical conformation at the temperature of the phase separation.

All our NMR results indicated small differences in the KC concentration of the isotropic and the nematic

* To whom correspondence should be addressed.
† Physical Chemistry 1, Lund University.
‡ Physical Chemistry, Umeå University.

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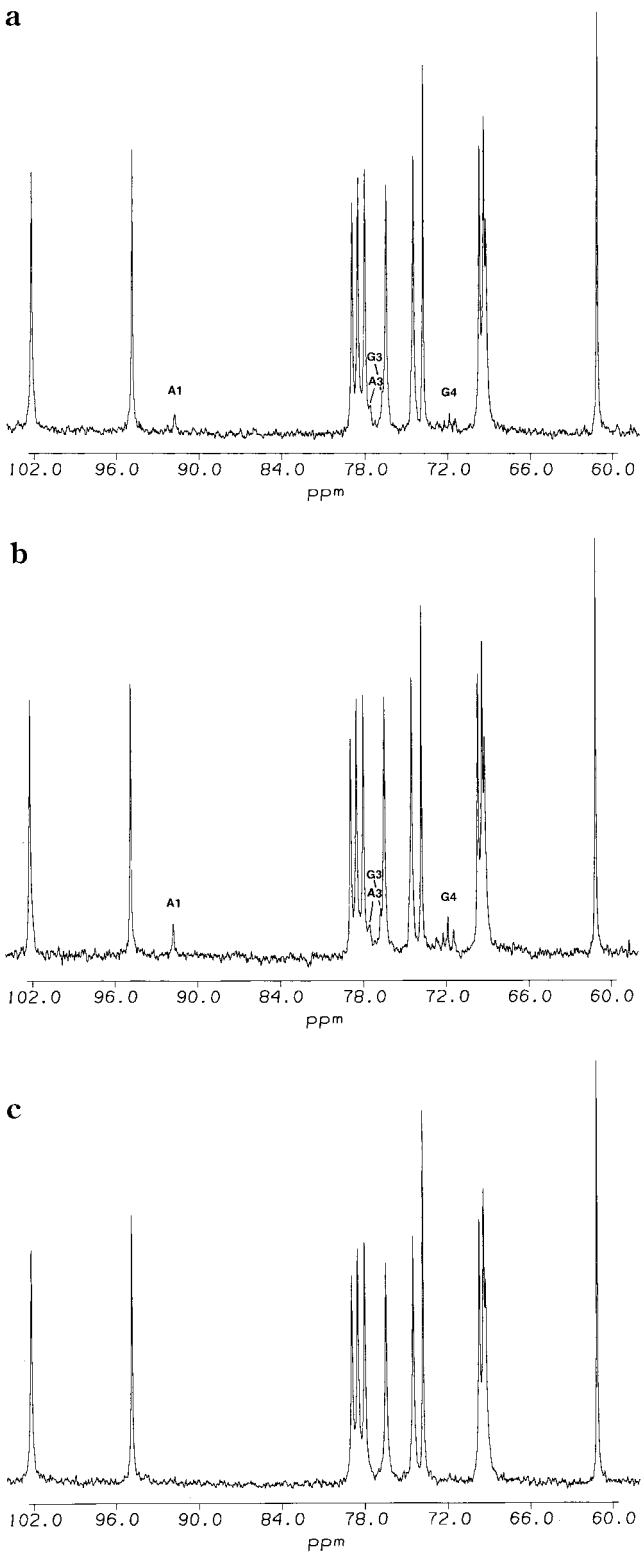

Figure 2. ^{13}C spectra at 150.85 MHz, 80 °C of the mother solution (a) and the separated isotropic (b) and nematic (c) phases of 6% KC in 0.1 M NaI. Peaks due to the IC contamination (assignments according to Greer et al.[12]) are indicated by the carbon numbers and the sugar units; A = 3,6-anhydrogalactopyranose and G = galactopyranose.

phases of KC, as expected for an isotropic−nematic phase equilibrium in a good solvent.[14−16] This was confirmed by elemental analysis (Table 1). Both the carbon analysis and the water content showed that the concentration of KC was only slightly higher (by ca. 0.6 wt %) in the nematic phase. Note, however, that the sulfur concentration was actually larger in the isotropic phase, which is consistent with an enrichment of IC (cf. Figure 1). The amounts of sulfur and carbon detected by elemental analysis correspond to molar ratios of 0.94 and 1.3 sulfur atoms per disaccharide unit in the nematic and isotropic phases, respectively, to be compared with the value of 1 for pure KC.

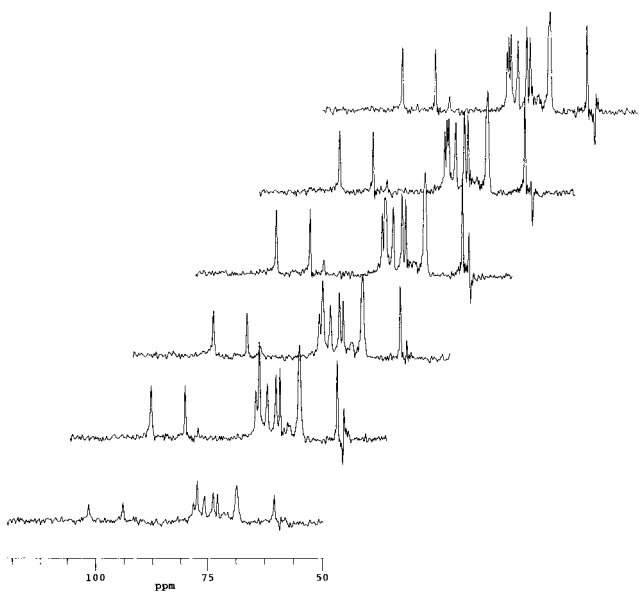

Figure 3. ^{13}C spectra at 100.7 MHz of the separated isotropic phase of 6% KC in a mixture of 0.08 M NaI and 0.02 M CsI recorded at the temperatures (from top to bottom) 75, 65, 55, 45, 35, and 25 °C.

Table 1. Elemental Analysis of the Equilibrium Phases of 6% κ-Carrageenan Dissolved in an Aqueous (10% D_2O) Solution Containing 0.08 M NaI, 0.02 M CsI

	contents (wt %)				
phase	C[a]	S[a]	water	Na	Cs
isotropic	22.6	6.5	92.5	0.59	0.3
nematic	24.8	5.2	91.8	0.60	0.4

[a] Expressed as weight percent of the solids.

Discussion. Our study demonstrates a novel way to physically remove the IC contamination in KC from *E. cottonii*, by its uneven partitioning in the isotropic/nematic two-phase system of KC. The IC contamination clearly behaves like a fraction of pure IC: It forms helices in the expected temperature interval, and it may be separated from virtually pure KC by physical methods. Our results thus confirm previous results showing,[3−6,10] at least for samples from the most common algal sources, that IC impurities in KC (and vice versa) mainly exist in separate molecules, rather than in "hybrid" chains[9] of mixed KC/IC character.

We find it quite remarkable that the exclusion of IC helices from the nematic phase of helical KC is so strong, given that the two carrageenans are so similar (also with respect to the proposed structures of the helices[17]), and furthermore, that the contents of all other components in the separating phases are so similar. The strong fractionation becomes even more puzzling in the light of our finding that other polysaccharides distribute much more evenly between the isotropic and the nematic phases of KC.[18] Interestingly, we have so far not succeeded in obtaining nematic solutions of pure IC, despite several attempts with both degraded and nondegraded samples in solutions of different salt compositions.

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