On the structure of κ/ι-hybrid carrageenans

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Abstract

The coil-to-helix transition and temperature dependence of the viscosity of commercial κ/ι-hybrid carrageenans produced by the red algae Sareothalia crispa, Mazzaella laminarioides, and Chondrus crispus were studied using rheometry and optical rotation. The structure of these κ/ι-hybrid carrageenans was determined by 1H and 13C NMR spectroscopy combined with monosaccharide composition analysis. The coil-to-helix transitions, measured by polarimetry and rheometry, of the κ/ι-hybrid carrageenans are significantly different from those of pure κ- and ι-carrageenan, and from hand-made mixtures thereof. This provides evidence that the κ/ι-hybrid carrageenans are mixed chains, containing both κ- and ι-repeating units. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Carrageenan; κ/ι-Hybrids; Polysaccharides; Coil-to-helix transition; Optical rotation; Rheometry; NMR; Structural analysis

1. Introduction

Polysaccharides are widely used in the food industry because they can favourably affect the texture of processed foods. They are used alone or in combination with proteins. Carrageenan is predominantly used for food applications, the dairy sector representing the largest part. In general, carrageenan serves as a gelling, stabilising and viscosity-building agent.

Carrageenans are a family of water-soluble, linear, sulfated galactans extracted from species of marine red algae, the Rhodophyceae. They are composed of alternating 3-linked β-D-galactopyranose (G-units) and 4-linked α-D-galactopyranose (D-units) or 4-linked 3,6-anhydrogalactose (A-units), forming the disaccharide repeating unit of carrageenans (see Fig. 1). The sulfated galactans are classified according to the presence of the 3,6-anhydrogalactose on the 4-linked residue and the position and number of sulfate groups. The most common types of carrageenans are traditionally called κ-, ι-, λ-, ν-, and µ-carrageenans. The rheological properties of the gelling carrageenans (κ and ι) are quite distinct: the κ-type forms gels that are hard, strong and brittle, whereas ι-carrageenan forms soft and weak gels. ν- and µ-Carrageenans are generally considered as the biological precursors of ι- and κ-carrageenan, respectively.
Fig. 1. Schematic representation of the different repeating units of carrageenans. The letter codes refer to the alternative nomenclature, as developed by Knutson et al.²

Industrially κ- and τ-carrageenans sometimes show behaviour (rheometric, spectroscopic) which suggests they are κ/τ-hybrid carrageenans. By 'κ/τ-hybrid carrageenans' we mean carrageenans that consist of both κ- and τ-repeating units as concluded from \(^1\)H or \(^\text{13C}\) NMR spectroscopy. The subject of the present work is to establish whether the κ/τ-hybrid carrageenans indeed consist of mixed chains (κ- and τ-repeating units present on the same chain, either blockwise or randomly ordered short sequences) or whether they are just mixtures of pure κ- and τ-chains. Knowing the detailed structure of commercial carrageenans is important with respect to the control and improvement of their often variable functionality as gelling and thickening agents.

The occurrence of κ/τ-hybrid carrageenans is quite common in nature, and they are produced in a whole range of different κ/τ-ratios by different species of the Gigartinaeae family.¹⁵ Seaweeds from this family have a life cycle with alternating gametophytic and sporophytic phases. During the gametophytic phase the κ/τ-hybrid carrageenans are produced, whereas during the sporophytic phase λ-carrageenan is produced. However, their detailed molecular structure has been a matter of debate for many years. Several attempts have been made to refine the knowledge of the molecular structure of these hybrids.

The carrageenans extracted from Gigartina skottsbergii have been studied using several techniques, e.g., KCl fractionation, IR and NMR spectroscopy, and methylation analysis.⁶-⁸ The results are that the cystocarpic system yields κ/τ-hybrid carrageenans and the tetrasporic system yields λ-carrageenan. NMR spectroscopy was also used by Stortz et al.⁹,¹⁰ to analyse the carrageenans extracted from Iridaeae undulosa. Carrageenans extracted form different Gymnogongrus, Ahnfeltia, and Stenogramme species are κ/τ-hybrid carrageenans with different κ/τ-ratios as reported by Furneaux and Miller.¹¹ IR spectroscopy was used to reveal the κ/τ-hybrid nature of several carrageenans extracted from Gigartinaeae and Hypneaceae species.¹² However, none of
the publications mentioned above, answers the question with respect to the molecular structure of the $\kappa$/$\iota$-hybrid carrageenans, i.e., a mixture of chains or a mixed-chain structure.

A more detailed study on the molecular structure of carrageenans was published by Rochas et al. They studied the structure of predominantly $\kappa$-carrageenan extracted from *E. cottonii* (*Kappaphycus alvarezii*) and concluded that it was a mixture of polysaccharides with $\kappa$-carrageenan as the major constituent and polymers with a large percentage of $\iota$-carrageenan and methylated galactans as minor constituents. Besides this high-molecular-weight carrageenan, *K. alvarezii* also contains a small amount of low-molecular-weight $\kappa$/$\iota$-hybrid carrageenan, which is only observed after cold extraction.

The physical properties of mixed $\kappa$- and $\iota$-carrageenan gels have been studied using different techniques. Parker et al. studied the rheology and fracture of mixed $\kappa$- and $\iota$-carrageenan gels and concluded that the two carrageenans gel separately at different temperatures. A differential scanning calorimetry (DSC) study by Ridout et al. confirmed this. Also the influence of small amounts of $\kappa$-carrageenan on the properties of $\iota$-carrageenan have been studied.

We used $^1$H and $^{13}$C NMR spectroscopy, combined with monosaccharide analysis, to reveal the molecular structure of two commercial $\kappa$/$\iota$-hybrid carrageenan samples. One was extracted from *Chondrus crispus*, and the other was extracted from a mixture of *Saccharothrix crispata* (75%) and *Mazaeilla laminarioides* (25%). Optical rotation measurements and viscometry were used to study the coil-to-helix behaviour of these hybrid samples. Finally KCl fractionation was used to discriminate between a mixture of chains and a mixed-chain structure in these samples.

2. Results

**Structure analysis.** — NMR spectra (Fig. 2) of the carrageenan samples were recorded at 60°C and pH 7–8, using sonicated material. $^1$H NMR spectra were interpreted using the chemical shift data for the anomic protons as reported by Ciancia et al. and Stortz et al. $^{13}$C NMR spectra were interpreted using the chemical shift data of carbon atoms as reported by Stortz and Cerezo and Ciancia et al. The chemical shifts for the most important carrageenans observed by us are only slightly different from those reported in literature.

The position and relative intensities of the signals of the anomic carbon atoms in the $^1$H and $^{13}$C NMR spectra were used to determine the composition of the carrageenan samples (Table 1). The galactose-3,6-anhydrogalactose ratio of the carrageenans was determined by anion-exchange chromatography (HPAEC) according to Jol et al., and the results of this analysis are included in Table 1. The hybrid carrageenan samples (CH021 and CH024) will be referred to as hybrid 50/50 and hybrid 70/30, even while their exact fractions of $\kappa$- and $\iota$-carrageenan differ from 50:50 and 70:30. Quantification of $^{13}$C NMR spectra was based on the intensities of the resonances of the anomic carbons. The $T_1$ values for the resonances of the anomic carbons of $\kappa$- and $\iota$-carrageenan were determined experimentally and turned out to be smaller than 0.45 s. For the $^{13}$C NMR spectra, an inter-pulse delay of 1.5 s was used, which allows a reasonable quantitative interpretation of the intensity of the anomic carbons. The assumption was made that the other carrageenan forms have comparable $T_1$ values.

No significant difference was observed between the ratios of intensities of the anomic carbon resonances in a spectrum of a sample containing both $\iota$- and $\kappa$-carrageenan, acquired using inverse gated decoupling and obtained with composite pulse decoupling. This demonstrates that the NOE enhancement is similar for these carbons. It is reasonable to assume that this holds true for all forms of carrageenan. The fact that the ratios of the different carrageenan forms deduced from $^{13}$C and $^1$H NMR spectra are very similar justifies the assumption mentioned above.

**Optical rotation.** — The optical rotation of the carrageenan samples as a function of temperature is given in Fig. 3. The coil-to-helix transition of $\kappa$- and $\iota$-carrageenan occurs at distinct temperatures. At the ionic strength...
Fig. 2. 13C (left) and 1H NMR (right) spectra of carrageenan samples: (A) κ-carrageenan (CH010), (B) λ-carrageenan (CH108), (C) κ-λ-hybrid carrageenan (CH021); (D) κ-λ-hybrid carrageenan (CH124).
Table 1
Composition of carrageenan samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Repeating unit composition based on $^{13}$C NMR spectroscopy (mol%)</th>
<th>Repeating unit composition based on $^1$H NMR spectroscopy (mol%)</th>
<th>Monosaccharide composition based on $^1$H NMR (mol%)</th>
<th>Monosaccharide composition based on HPAEC (mol%)</th>
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<tr>
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<td>$\iota$</td>
<td>$\mu$ or $\nu$</td>
<td>$\kappa$</td>
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<tr>
<td>CH025</td>
<td>91</td>
<td>9</td>
<td></td>
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</table>

$^a$HPAEC, high-performance anion-exchange chromatography.
$^b$The anemic carbon resonances of $\mu$- and $\nu$-carrageenan (98.4 and 98.7 ppm) are too close to each other to decide between the two carrageenans.
$^c$Not identified.
Fig. 3. Optical rotation as a function of temperature for the carrageenan samples (0.1% w/w in 0.2 M NaCl): • t-carrageenan (CH016); ▪ κ-carrageenan (CH018); ▲ carrageenan containing 31% t-units/48% κ-units/21% v-units (CH020); ▼ κ/v-hybrid carrageenan (CH021; 50% κ-units/50% t-units); ■ v-containing t-carrageenan (CH022); + κ/ν/hybrid carrageenan (CH024: 70% κ-units/30% t-units).

Fig. 4. Optical rotation as a function of temperature for carrageenans (0.1% w/w in 0.2 M NaCl): • t-carrageenan (CH016); ▪ κ-carrageenan (CH018); ▲ hand-made mixture of pure κ- and pure t-carrageenan (50% CH016/50% CH018); ▼ κ/ν-hybrid carrageenan (CH021: 50% κ-units/50% t-units).

from pure κ- and t-carrageenan samples CH018 and CH016, respectively.

Fig. 4 shows that the hand-made mixture of κ- and t-carrageenan has two separate coil-to-helix transitions: one for κ-carrageenan at 23 °C and one for the t-carrageenan at 58 °C. In the case of the κ/ν-hybrid carrageenan (CH021), no clear coil-to-helix transition was observed upon cooling. In the random coil conformation, all carrageenans have a very similar specific optical rotation (0.07° (dm mM)). Upon cooling the optical rotation of the hybrid carrageenan starts to increase around 40 °C, and the increase in specific optical rotation at 10 °C is only half of that of the pure carrageenans and the κ- and t-mixture. Also the κ/ν-hybrid CH024 (70% κ-units and 30% v-units) does not show a clear coil-to-helix transition (see Fig. 3).

Viscometry.—Fig. 5 shows the specific viscosity as a function of temperature for different carrageenans. In the random coil conformation, κ- and t-carrageenan have the same specific viscosity (0.32 L/mm mol), whereas the specific viscosity of the hybrid carrageenan (CH021) is slightly lower (0.26 L/mm mol). It turns out that the hand-made mixture of κ- and t-carrageenan shows two separate transitions in the viscosity with decreasing tempera-

chosen (0.2 M NaCl), the onset temperatures of the coil-to-helix transition are 23 and 58 °C for κ- and t-carrageenan, respectively. Also the shape of the curve is characteristic for each type of carrageenan. The transition temperatures, as well as the shape of the curves, are in good agreement with the results of Nilsson and Piculell13 and Viebke et al.20 Another observation, which falls beyond the scope of the present work, is made here: the coil-to-helix transition is dramatically changed when precursor units (ν-carrageenan in the samples CH020 and CH022) are present. These so-called kinking units21 cause an interruption of the regular structure. Small amounts of these precursors suffice to prevent the formation of a regular helix conformation.

As shown in Fig. 3, carrageenans, from NMR results suspected to be κ/ν-hybrids, do not exhibit a sharp coil-to-helix transition in the optical rotation measurement. We studied the coil-to-helix transition of these suspected hybrid samples in more detail and compared the coil-to-helix transition of the hybrid carrageenan CH021 (50% κ-units and 50% t-units) with that of a hand-made mixture with an identical monomeric composition prepared
Discussion

Structure analysis.—The aim of this study on \( \kappa / \iota \)-hybrid carrageenans extracted from the red seaweed, *C. crispus*, and a mixture of the seaweeds, *S. crispata* and *M. laminarioides* (both *Iridaea* species), was to reveal the molecular structure of these carrageenans. The overall composition of the carrageenan samples (Table 1) was determined with \(^1\)H and \(^{13}\)C NMR spectroscopy. The position and intensity of the anomeric signals in \(^{13}\)C NMR (\(90-110\) ppm) and \(^1\)H NMR (\(5.0-5.6\) ppm) accurately reflect the carrageenan monomeric composition, both with respect to the types of repeating units present and to their amounts. Determination of the monosaccharide composition with anion-exchange chromatography gave the ratio between galactose and 3,6-anhydrogalactose. The idealised carrageenan structures (Fig. 1) predict a galactose-3,6-anhydrogalactose ratio of 50:50 for the \( \kappa \)- and \( \iota \)-repeating units. Precursor containing samples are characterised by an excess of galactose.

Several remarks can be made concerning the data in Table 1. The \( \kappa \)- and \( \iota \)-carrageenan samples contained impurities of \( \iota \)- and \( \kappa \)-carrageenan, respectively. The occurrence of impurities in carrageenans extracted from *K. alvarezii* and *E. denticulatum* is quite common.\(^{13,18}\) The galactose-3,6-anhydrogalactose ratio of the alkali-treated samples varied from 45:55 (CH025) to 53:47 (CH021), which is in reasonable agreement with the expected 50:50.

The pairs of samples CH020/CH021 (extracted from the *Iridaea* species) and CH022/CH023 (extracted from *E. denticulatum*) showed that alkaline treatment of carrageenan samples reduces the amount of precursor units. The precursor content (calculated from the excess of galactose compared to 3,6-anhydrogalactose) of these samples determined with the monosaccharide composition analysis is 34 and 14\%, respectively, for CH020 and

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Table 2 summarises the composition of the fractions obtained by KCl fractionation.

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KCI fractionation.—We investigated the KCl fractionation of the \( \kappa / \iota \)-hybrid carrageenans (CH021 and CH024) using the method reported by Rochas et al.\(^{13}\) Hand-made mixtures of pure \( \kappa \)- and \( \iota \)-carrageenan were included as blanks. Phase separation was observed for the hand-made mixtures of \( \kappa \)- and \( \iota \)-carrageenan (50/50 and 70/30 mol/mol). Upon centrifugation a gel phase and a supernatant were formed. After dialysis the two layers were analysed by \(^1\)H NMR spectroscopy (Fig. 6). The \( \kappa / \iota \)-hybrid carrageenans did not undergo a phase separation upon centrifugation. In the case of CH021, a clear homogeneous viscous solution was obtained. Samples for \(^1\)H NMR analysis were taken from the top and bottom of the solution. In the case of CH024 centrifugation resulted in a heterogeneous viscous solution containing small gel particles. Samples were taken from the top of the solution (poor in gel parts) and from the bottom (rich in gel parts). The incomplete separation of the hybrid samples into a gel and supernatant phase precluded the determination of the yields of the two phases.
Optical rotation and viscometry. — The gelation on cooling of κ- or τ-carrageenan solutions is generally accepted to proceed in two steps. The first step is the coil-to-helix transition, from a disordered (random coil) to an ordered (helical) conformation. The temperature of the coil-to-helix transition is strongly dependent on the type and structure of the carrageenan under study, as well as on the type and concentration of salt added to the carrageenan solution.
Composition of carrageenan fractions obtained by KCl fractionation of hand-made mixtures of pure κ- and τ-carrageenan and κ/τ-hybrid samples as determined by 1H NMR spectroscopy

<table>
<thead>
<tr>
<th>Sample</th>
<th>Starting mixture</th>
<th>Gel or bottom layer</th>
<th>Supernatant or top layer</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>κ (%)</td>
<td>τ (%)</td>
</tr>
<tr>
<td>A</td>
<td>Mixture 50% κ/50% τ</td>
<td>55</td>
<td>45</td>
</tr>
<tr>
<td>B</td>
<td>κ/τ-Hybrid CH021</td>
<td>49</td>
<td>51</td>
</tr>
<tr>
<td>C</td>
<td>Mixture 70% κ/30% τ</td>
<td>72</td>
<td>28</td>
</tr>
<tr>
<td>D</td>
<td>κ/τ-Hybrid CH024</td>
<td>66</td>
<td>34</td>
</tr>
</tbody>
</table>

The changes in the increase in viscosity of κ- and τ-carrageenan occur at a somewhat lower temperature (around 2–3 °C) than their coil-to-helix transition as observed with optical rotation measurements. This is in good agreement with the general accepted idea that the coil-to-helix transition occurs before association of helices and gelation takes place. The separate coil-to-helix transitions as observed for the hand-made mixtures of pure κ- and pure τ-carrageenan in polarimetry and viscometry is in agreement with the observations of Parker et al. and Ridout et al. who observed a two-step gelation of mixed κ- and τ-carrageenan gels by DSC and rheological measurements, respectively. However, these observations are in contrast with the results of Rochas et al., who observed a synergistic effect in a mixture of κ- and τ-carrageenan. In their optical rotation measurement an increase in transition temperature was observed for a mixture of κ- and τ-carrageenan; unfortunately, they did not mention the salt concentration used for this measurement.

The κ/τ-hybrid carrageenans did not show a clear coil-to-helix transition (Figs. 3 and 4) or any sudden change in the increase in viscosity with decreasing temperature (Fig. 5). The possibility of hybrids being a mixture of separate κ- and τ-chains is therefore ruled out because this situation would result in two separate coil-to-helix transitions, as observed for the hand-made mixtures. Two further explanations can be suggested: hybrids consist of chains containing both κ- and τ-repeating units, or hybrids consist of two types of chains, κ and τ, both containing precursor units (μ and ν) that occur below the detection level of 13C NMR detection, but which destroy the helix-forming capacity. We tested the latter explanation by using an alkaline treatment (0.2 M NaOH at 100 °C for 2 h), which is sufficient to convert the precursor containing sample CH022 into τ-carrageenan (CH023). Alkaline treatment of the hybrid samples CH021 and CH024 did not influence their optical rotation versus temperature curves. Therefore, the possibility of precursor units disturbing the coil-to-helix transition of these κ/τ-hybrid carrageenans can be ruled out.

Consequently, the most probable explanation for the absence of transitions in the case of the κ/τ-hybrids is the occurrence of mixed chains in the κ/τ-hybrid carrageenan samples. Chains that are composed of both κ- and τ-repeating units are expected to contain kinking sequences (κ-units adjacent to τ-units) that prevent a regular helix formation.
**KCl fractionation.**—The results were that hand-made solutions of \( \kappa \)- and \( \tau \)-carrageenan could be separated in a gelled phase of predominantly \( \kappa \)-carrageenan and a liquid phase of predominantly \( \tau \)-carrageenan by choosing salt conditions in which \( \kappa \)-carrageenan forms a gel and \( \tau \)-carrageenan does not. The same conditions for the presumed hybrids resulted in no separation, implying that \( \kappa \)- and \( \tau \)-repeating units, detected by NMR spectroscopy, occur on the same chain. The presence or absence of KCl fractionation is further confirmed by \( ^1H \) NMR analysis of the top and bottom layers of the sample tubes after centrifugation. The \( ^1H \) NMR spectra of the mixture before fractionation (samples were taken after mixing the KCl and carrageenan solutions; spectra I in Fig. 6) show that the signals of the anomeric protons of the 3-linked \( \beta \)-D-galactopyranose of the hand-made mixtures (A1 and C1) and the \( \kappa/\tau \)-hybrid carrageenans (B1 and D1) gave the same ratio between \( \kappa \)- and \( \tau \)-repeating units. From the spectra of the hand-made mixtures (A and C), it is clearly seen that the gel phase (the bottom layer after centrifugation) is enriched in \( \tau \)-carrageenan and the supernatant in \( \kappa \)-carrageenan. The gel phase contained 87\% \( \kappa \)-carrageenan in both cases (see Table 2), whereas the supernatant contained 94 and 86\% \( \tau \)-carrageenan for, respectively, the 50/50 and 70/30 mixture. In contrast, the spectra of the top and bottom layer of the \( \kappa/\tau \)-hybrid (CH021) are identical to the starting material (see spectra B and D). The amount of \( \kappa \)-units is 49\% in all phases (see Table 2). In the case of hybrid (CH024) the excess of \( \kappa \)-repeating units in the starting material (66\%) resulted in a bottom layer that is slightly enriched in \( \kappa \)-units (69\%).

The results obtained with the KCl fractionation of \( \kappa/\tau \)-hybrid carrageenans are in agreement with the results obtained by rheology and measurements of the optical rotation. In hand-made mixtures of pure \( \kappa \)- and pure \( \tau \)-carrageenan, the two types of carrageenan behave independently from each other. They have their own coil-to-helix transition temperature, and they gel at different temperatures.

In contrast, the \( \kappa/\tau \)-hybrid carrageenans behave differently from the hand-made mixtures of pure \( \kappa \)- and pure \( \tau \)-carrageenan: no clear coil-to-helix transition and discontinuous increase in viscosity is observed and also no separation of \( \kappa \)-chains and \( \tau \)-chain takes place upon KCl fractionation. From these results, we conclude that the \( \kappa/\tau \)-hybrid carrageenans are mixed chains containing both \( \kappa \)- and \( \tau \)-repeating units. These mixed chains may consist of randomly ordered \( \kappa \)- and \( \tau \)-repeating units, or blockwise ordered \( \kappa \)- and \( \tau \)-regions. A detailed comparison between the \(^{13}\)C NMR spectra of the hybrid carrageenans and a \(^{13}\)C NMR spectrum of a hand-made mixture of pure \( \kappa \)- and \( \tau \)-carrageenan showed no differences between these spectra. This indicates that the hybrid carrageenans are composed of long blocks of \( \kappa \)- and \( \tau \)-repeating units. However, distinguishing these structures requires specific partial enzymatic degradation, as reported by Rochas et al.,\(^{13}\) which is beyond the scope of the present work.

**4. Experimental**

**Materials.**—Carrageenan samples were obtained from Hercules, Inc. (Copenhagen Pectin A/S, Lille Skensved, Denmark) and SKW Biosystems (Boulogne Billancourt Cedex, France). Table 3 presents an overview of the sample numbers used throughout this paper and the sample description given by the manufacturer. Note that in this paper the taxonomically correct names *Kappaphycus alvarezii* and *Eucheuma denticulatum* are used instead of the names for \( \kappa \)- and \( \tau \)-producing seaweeds, *Eucheuma cottonii* and *Eucheuma spinosum*, respectively, which are often used by commercial suppliers as well as in scientific reports.

**Sample preparation.**—All carrageenan samples were converted to the \( Na^+ \) form before analysis, by ion exchange (Dowex MSC-1 resin) at 85 °C. Care was taken to avoid acidification of the carrageenan samples at elevated temperature, because this causes rapid hydrolysis of the carrageenans. Therefore, an alkaline NaCl solution was used to obtain the \( Na^+ \)-form of the resin. The resin (20 g) was washed with water (500 mL), HCl (1.0 M; 500 mL), water (1500 mL), brought into the \( Na^+ \)-form with an NaCl solution (1.0
Table 3
Origin of the carrageenan samples

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</tbody>
</table>

a Hercules Inc., Copenhagen Pectin A/S, DK-4623 Lille Skensved, Denmark; SKW, SKW Biosystems, 4 Place des Ailes, 92642 Boulogne Billancourt Cedex, France.
b Treatment: nt, not treated; t, treated with alkali; u, unknown.
c Cation contents are expressed in mol% of total cations, determined by atomic absorption spectroscopy.
d Broad leaf Iridae.
e Narrow leaf Iridae.

M; 500 mL; containing 0.05 M NaOH), and finally washed with water (500 mL). Carrageenan (1.5 g) was dissolved in water (500 mL). After ion exchange, the volume was reduced to 100 mL by rotary evaporation (60 °C; 50 mmHg) and lyophilised. During ion exchange, the pH of the carrageenan solution was never below pH 7.5. SEC-MALLS analysis showed that the ion-exchange procedure resulted in a reduction of the molecular weight of the carrageenan by only 3%. The efficiency of the procedure was controlled by atomic absorption spectroscopy: the sodium content exceeded 99 mol% of the total amount of cations. Sample concentrations are given in mM disaccharide, using the composition of the carrageenan samples as given in Table 3.

13C NMR spectroscopy.—The spectra were taken on a Bruker AM 400 spectrometer operating at 100.6 MHz. Typically 25,000 scans were accumulated at 60 °C using a 90° pulse, an inter-pulse delay of 1.5 s and composite pulse decoupling. The experimentally determined $T_1$ values for the anomic carbons of κ- and τ-carrageenan were shorter than 0.45 s. Spectra taken with continuous and with inverse gated decoupling showed the same κ/τ ratios for the anomic carbons, indicating that the NOE enhancement for the resonances of the anomic carbons of both types of carrageenans is comparable, which is in accordance with literature. This means that the intensity of the resonances of the anomic carbons can be used for the determination of the composition of carrageenan samples. An external reference was used consisting of 1.9 M MeOH (51.75 ppm) and 0.18 M 3-trimethylsilylpropionic acid-d$_4$ sodium salt (0 ppm) in D$_2$O.

The sample preparation for the 13C NMR spectra was as follows. A solution of 0.5% w/w Na+-form carrageenan in water was prepared at 80 °C. This solution was sonicated for 1 h in melting ice (Heat Systems XL 2020 sonicator, 12 mm tip, power 475 W, frequency 20 kHz). If required the solution was centrifuged at 56 °C to remove insoluble material and then lyophilised. For the NMR experiments the material was dissolved in D$_2$O up to a concentration of 5–10% w/w, and the pH was adjusted to 7–8 by addition of 20 mM Na$_2$HPO$_4$.

1H NMR spectroscopy.—The 1H NMR spectra were taken on a Bruker DRX 600 spectrometer operating at 600.13 MHz and on a Bruker AM 400 spectrometer operating at 400.13 MHz. The spectra were recorded at 60 °C. Typically 64 scans were taken with an interpulse delay of 5 s (experimentally determined $T_1$ values for the resonances of κ- and τ-carrageenan were smaller than 1.5 s). The spectra were referenced using the chemical shift of the HDO resonance.
The sample preparation for the $^1$H NMR experiments involved solving the Na$^+$-form carrageenan sample in D$_2$O (containing 20 mM Na$_2$HPO$_4$) up to a concentration of 0.1–0.5% w/w at 80 °C, sonication for 1 h with a 3 mm tip at a power of 230W and centrifugation.

Galactose-3,6-anhydrogalactose ratio.—Samples for the determination of the galactose-3,6-anhydrogalactose ratio were prepared according to Jol et al.18 by an acid-catalysed hydrolysis under reducing conditions using a methylmorpholine-borane complex, followed by high-performance anion-exchange chromatography (HPAEC) of the formed alditols. The HPAEC instrument consisted of a Waters 600 E controlled HPLC pump, a CarboPac PA1 column (4 x 250 mm from Dionex), and an electrochemical detector model 400 EG&G in pAD mode (gold electrode: $E_1 = 100$ mV; $t_1 = 0.4$ s; $E_2 = 600$ mV; $t_2 = 0.3$ s; $E_3 = -800$ mV; $t_3 = 0.3$ s) coupled to a Spectra Physics SP4270 Integrator. An NaOH solution (37.5 mM) was used as eluent at a flow rate of 0.4 mL/min.

Optical rotation measurements.—The coil-to-helix transitions (cooling curves) were monitored by optical rotation at 365 nm on a Perkin-Elmer 241 polarimeter in a jacketed cell with a 10-cm path length. The temperature was controlled with a circulating water bath. Specific optical rotation values are given in degrees/(dm mM) (moles of repeating unit based on the composition of the samples as indicated in Table 3). Samples were prepared by dissolving (at 80 °C) carrageenan (40 mg; 0.1% w/w) in a NaCl solution (40 mL; 0.2 M).

Viscosity measurements.—Viscosity versus temperature curves were measured with an Ubbelohde viscometer ($\phi_{capillary\ tube} = 0.63$ mm; $V = 20$ mL). Samples were prepared by dissolving (at 80 °C) carrageenan (30 mg; 0.1% w/w) in a NaCl solution (30 mL; 0.2 M). The viscometer was loaded with the hot carrageenan solution (80 °C) and placed in a waterbath (65 °C) and equilibrated for 30 min. The measurements were started by lowering the setpoint to 10 °C and allowing the water bath to cool down (initial rate: 0.2°C/min), both flow time and temperature were recorded.

Alkaline treatment.—Carrageenan (0.3 g) was dissolved in water (45 mL) and heated to 100 °C. An NaOH solution (2.0 M; 5 mL; final concentration 0.2 M) was added, and the mixture was heated at 100 °C for 2 h. To the reaction mixture a cold NaCl solution (0 °C; 0.4 M; 50 mL) and a cold HCl solution (0 °C; 0.2 M; 50 mL) were added. The carrageenan samples (final concentration 0.2% w/w in 0.2 M NaCl) were analysed by optical rotation measurements without further purification.

KCl fractionation.—$\kappa$/$\lambda$-Hybrids and hand-made mixtures of pure $\kappa$- and pure $\lambda$-carrageenan were subjected to KCl fractionation according to Rochas et al.13 To a solution of crude carrageenan as received from the manufacturer (200 mL; 0.5% w/w) a KCl solution (300 mL; 50 mM) was added at rt to obtain a salt concentration and a final carbohydrate concentration of 30 mM and 0.2% w/w, respectively. After stirring for 48 h at rt, the mixture was centrifuged for 120 min at 9600 g at 15 °C. When possible the gel phase and supernatant were separated, dialysed against an NaCl solution (0.1 M, two times) and water (two times), lyophilised and analysed by $^1$H NMR spectroscopy.

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