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Molecular characteristics and gelling properties of carrageenan family 2. Tri-sulfated and tetra-sulfated carrageenans

Thuy T. T. Thành, Hidekazu Yasunaga, Ryo Takano, Hiroshi Urakawa, Kanji Kajiwara (✉)

Kyoto Institute of Technology, Kyoto, Sakyo-ku, Matsugasaki, 606-8585 Japan
e-mail: kajiwara@ipc.kit.ac.jp, Fax: +81-75-724-7564

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Summary

New series of tri-sulfated (τ-carrageenan) and tetra-sulfated (ρ-carrageenan) carrageenans were prepared by the sulfating reaction from ι-carrageenan. The chain conformations of new carrageenans in aqueous solutions were investigated in the presence of various metal salts by means of static/dynamic light scattering and small-angle X-ray scattering, and their conformational characteristics were discussed with respect to the gelation behaviour. Although the overall conformation of newly prepared carrageenans is specified as a random coil, the local chain retains a rigid-rod-like structure by forming a helical conformation. The sulfate groups on C2 of (1→3)-linked β-D-galactoses were found primarily responsible to prevent gelation.

Introduction

Carrageenans are the general term for sulfated polysaccharides extracted from red seaweed, and classified as kappa, iota, alpha, beta, lambda and theta according to the number and position of sulfate groups in the repeating units[1] as shown in Figure 1. Carrageenans are basically composed of alternating (1→3)-linked β-D-galactose and (1→4)-linked α-D-galactose, where (1→3)-linked β-D-galactoses are partially sulfated at the positions of C2 and/or C4 and (1→4)-linked α-D-galactoses are occasionally 3,6-anhydrided[2]. The reaction route is now well established to sulfated or desulfated carrageenans regioselectively. In this report, we prepare new series of tri-sulfated (τ-carrageenan) and tetra-sulfated (ρ-carrageenan) carrageenans by the sulfating reaction with H_2SO_4-*N,N'*-dicyclohexylcarbodiimide (see Figure 2 for the reaction scheme) from ι-carrageenan in compliment to β- and θ-carrageenan prepared in the preceding paper[3], and their dilute solution properties are examined by means of static/dynamic light scattering and small-angle X-ray scattering. The gelling properties are also examined and the effect of sulfate groups on gelation is discussed to understand the mechanism of gelation from the viewpoint of the conformational characteristics of tri-sulfated and tetra-sulfated carrageenans.

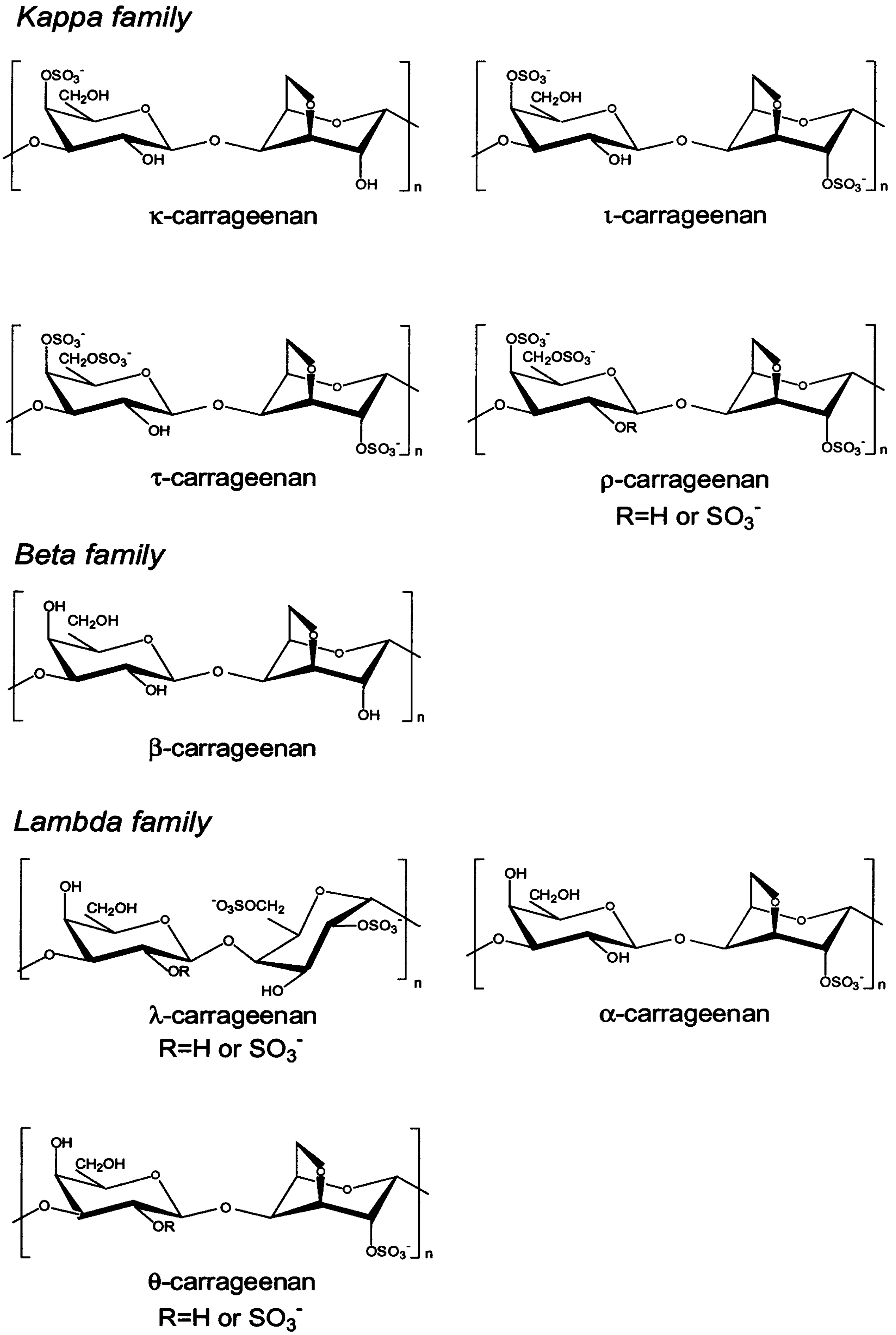

Figure 1. Chemical structure of the carrageenan family

Experimental

(a)Preparation of tri- and tetra-sulfated carrageenans

ι-carrageenan was purchased from Sigma. τ- (tri-sulfated) and ρ- (tetra-sulfated) carrageenans were prepared by sulfation of ι-carrageenan with sulfuric acid and *N,N'*-dicyclohexylcarbodiimide (DCC, Nacalai Tesque) used as a condensation reagent [4,5]. Here the term of τ- or ρ-carrageenan is adopted conventionally by avoiding the terminology of the traditionally available carrageenan family[6]. The aqueous solution of ι-carrageenan (Na salt) was applied to a column of Amberlite IR-120 (H-form), and the eluate was neutralized with pyridine and lyophilized. The resulting pyridine salt dissolved in DMF was mixed with DCC dissolved in DMF. After the solution was cooled to 0°C and mixed with H_2SO_4 / DMF solution, the mixture was stirred for 15 min at 0°C under N_2 atmosphere. The resulting mixture was poured onto crushed ice, neutralized with NaOH and dialyzed in Milli-Q water. The dialyzate was filtered with Celite 500 and centrifuged to remove dicyclohexylurea precipitate derived from DCC, and then the resulting polysaccharide was lyophilized. By controlling the amount of sulfuric acid added, τ- or ρ-carrageenan can be obtained (see the reaction scheme). The sulfation at C6 position of (1→3)-linked β-D-galactose residue was predominant in both of cases[4,5].

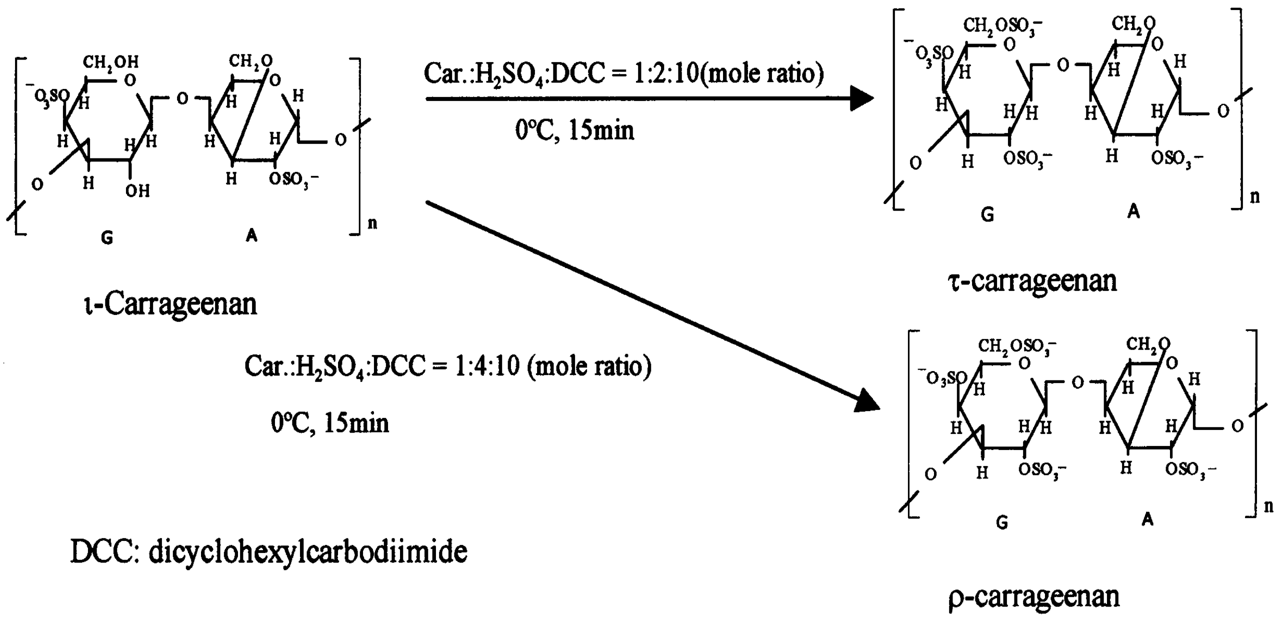

Figure 2. Reaction scheme of ι-carrageenan to τ- and ρ-carrageenan, where G and A denote the (1→3)-linked β-D-galactose and (1→4)-linked (3,6-anhydrousd) α-D-galactose, respectively.

(b)Molecular characterization

^{13}C NMR spectroscopy

The chemical structure of the obtained carrageenans was checked by ^{13}C NMR[7]. ^{13}C NMR measurements for the samples (3w% of D_2O solution) were performed at 80°C with a Bruker AC300 operating at 75.4 MHz. ^{13}C chemical shifts were calibrated through the internal methanol peak (49.3 ppm) with respect to tetramethylsilane. The chemical shifts are assigned as summarized in Table 1.

Table 1: Chemical shift$^{(a)}$ assignment in ^{13}C-NMR

Residue	(1→3)-linked β-D-galactose (G)						(1→4)-linked (3,6-anhydrided) α-D-galactose (A)					
Position	C1	C2	C3	C4	C5	C6	C1	C2	C3	C4	C5	C6
τ	102.8	69.3$^{(b)}$	76.6	71.9	72.6	68.0	91.8	74.8	78.2	79.2	77.0	69.3$^{(b)}$
ρ	100.5	77.0$^{(b)}$	73.6$^{(b)}$	73.6$^{(b)}$	72.4	67.9	94.0	74.5	77.7	78.6	77.0$^{(b)}$	69.5

(a) Chemical shift in ppm
(b) Overlapped with other signal

Chemical analysis

The number of sulfate groups per repeating disaccharide unit was determined by conductometric titration with NaOH aqueous standard solution for H-form carrageenan treated by a cation-exchange resin.

The ratio of anhydrous type in (1→4)-linked α-D-galactose unit of the carrageenans was determined by gas-liquid chromatography (GLC) using a Shimadzu chromatograph GC-8A, equipped with a frame ionization detector[8]. The carrageenans were hydrolyzed with sulfuric acid and $NaBH_4$[8,9]] and methylated in pyridine-acetic anhydride solution for GLC. Xylitol were used as the GLC internal standard. The chemical characteristics of the obtained carrageenans were summarized in Table 2.

Table 2: Chemical and molecular characteristics

Carrageenan Type	Gal-Gal / Gal-Angal (molar ratio)$^{(a)}$	Sulfate content$^{(b)}$	$M_w \times 10^{-3}$ / g mol^{-1}	R_g / nm	R_h / nm	ρ (=R_g/R_h)	B
τ	1: 18	2.50	492	63.1	38.5	1.64	0.125
ρ	1: 18	3.11	390	54.4	31.3	1.74	0.096
λ	3 : 1	2.20	2399	109	118	0.92	0.053[10]
θ	1 : 4	1.37	408	73.5	30.1	2.44	0.083[10]
κ	1 : 15	0.92	371	66.8	29.7	2.25	0.110[12]

(a) Gal and Angal denote the hydrated and anhydrated galactose residues, respectively
(b) mole H^+ / mole disaccharide

Viscosity measurements and flexibility parameter B

Viscosity measurements were carried out on a capillary viscometer at 25°C. The intrinsic viscosities were evaluated by extrapolating the viscosities at finite polymer concentrations to zero concentration according to the combined Huggins [$(\eta_{rel}-1)/c$ versus c] and Kraemer [$\ln\eta_{rel}/c$ versus c] procedure.

The intrinsic viscosities [η] of τ- and ρ-carrageenan were measured at different concentrations of added sodium salt in order to evaluate the flexibility parameter B[10,11,12]. The B value is defined as

$$B = S/([\eta])^{1.3} \tag{1}$$

at I = 0.1 where I and S denote the ionic strength and the slope of $[\eta]$ plotted against $I^{-1/2}$, respectively. The B value is considered as a measure of the resistance of polyelectrolytes to respond to the change in salt concentration, and can vary from 0.004 (a rigid rod) to over 0.24 (a random coil)[12]. The evaluated B values are summarized in Table 2. The intrinsic viscosities at I = 0.1 of the present τ- and ρ-carrageenan were evaluated as 0.327 ml/mg and 0.189 ml/mg, respectively.

Electromagnetic wave scattering

Static and dynamic light scattering was observed from 0. 1M NaCl aqueous solutions of carrageenans in the angular range from 35° to 150° in every 5° by an ALV Spectrometer 5000 equipped with a He-Ne laser (λ_0 = 633 nm). The refractive index increment dn/dc at λ_0 = 633 nm was determined by a Brice-Phoenix differential refractometer. Weight-average molecular weights M_w, radii of gyration R_g and hydrodynamic radii R_h were obtained by conventional and dynamic Zimm plots[13] as summarized in Table 2. Here a ρ value is defined as the ratio of the radius of gyration to the hydrodynamic radius ($\rho \equiv R_g/R_h$). The ρ value provides a measure on the shape of a solute molecule[14].

Small-angle X-ray scattering (SAXS) measurements were performed by an SAXES installed at BL-10C of the Photon Factory (Tsukuba, Japan) on aqueous solutions of τ- and ρ-carrageenan with or without salt at 5°C and 60°C. The SAXS intensities were accumulated for total 600s. The results were analyzed conventionally as described in the *Results and discussion* section.

Gelation behaviour

Gelation of τ- and ρ-carrageenan in alkali- and alkaline-earth-salt aqueous solutions was examined at 5°C and 60°C. Gel was identified by eye inspection when the sample solution would no longer flow in an inverted sample tube. The results are summarized in Table 3, together with the cross-sectional radius of gyration estimated from the SAXS measurements.

Table 3: Cross-sectional radius of gyration and gelling behaviour

Carrageenan (concentration)	Added salt	R_{gc} (nm)		Gel or sol	
		5°C	60°C	5°C	60°C
τ (C_p=1.5%)	0.1M NaCl	0.50	0.49	sol	sol
	0.1M KCl	0.48	0.47	sol	sol
	0.5M KCl	0.51	0.47	sol (gel(a))	sol
	0.1M $CaCl_2$	0.55	0.54	sol	sol
τ (C_p= 3%)	0.5M KCl	0.60	0.47	gel	sol
ρ (C_p= 1.5%)	0.1M NaCl	0.50	0.48	sol	sol
	0.1M KCl	0.50	0.49	sol	sol
	0.1M $CaCl_2$	0.56	0.56	sol	sol
ρ (C_p= 3%)	0.5M KCl	(b)	0.49	sol	sol

(a) Judged from the scattering profile in the Kratky plots

(b) The sample was destroyed by X-ray

Although neither τ- nor ρ-carrageenan forms gel in 0.1 M salt solutions, τ-carrageenan exhibits the symptom of gelation by increasing the salt concentration. For example, τ-carrageenan forms gel in 0.5 M KCl aqueous solution at 5°C by increasing the polymer concentration to 3 %, whereas the 1.5 % solution shows the tendency of gelation in the scattering profile at 5°C as observed from the sharp upturn at $q \rightarrow 0$ in the Kratky plots[15] (see Figure 3).

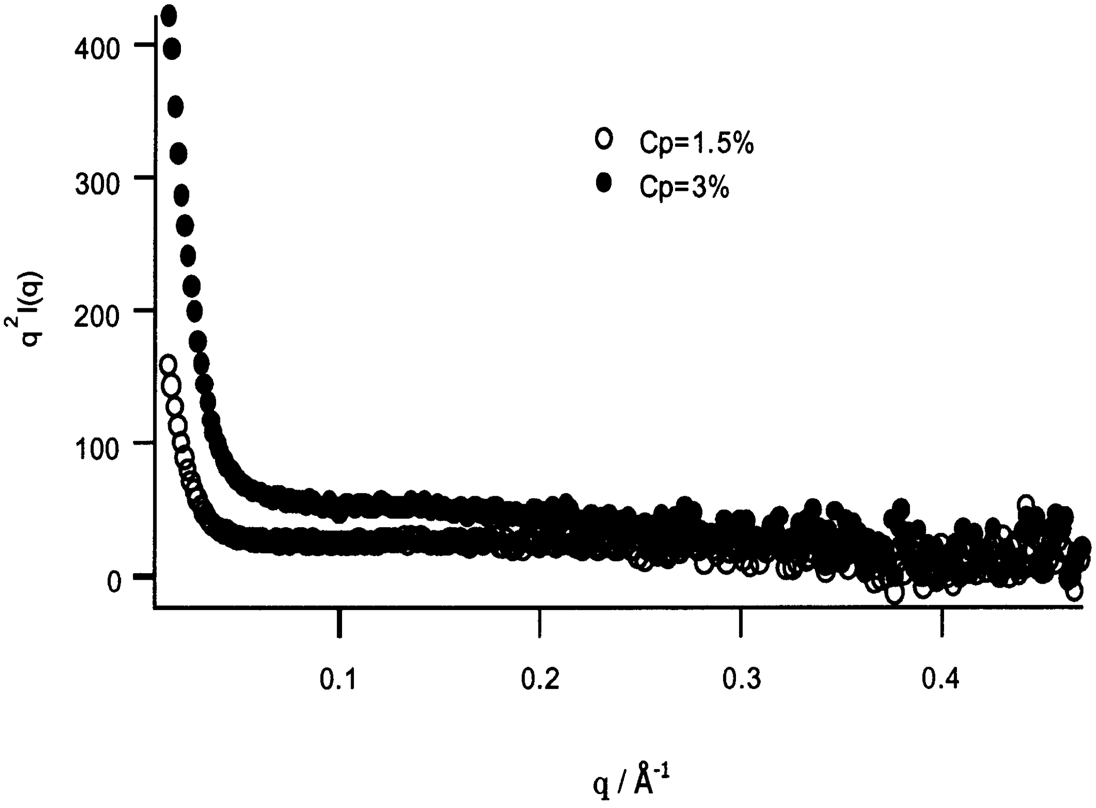

Figure 3. Kratky plots for small-angle X-ray scattering from τ-carrageenan in 0.5 M KCl aqueous solution at 5°C

Results and discussion

The present results of light scattering from 0. 1 M NaCl aqueous solution at 25°C indicate that the conformation of τ- and ρ-carrageenan would be classified as a random coil as the ρ values are in the range of 1.6 to 1.7[14]. In the preceding paper[3], κ-, ι- and θ-carrageenans were found to possess relatively large ρ ($=R_g/R_h$) values, indicating the chains are relatively stiff and rod-like. On the contrary, λ-carrageenan was found to possess a small ρ value, suggesting a relatively compact structure. β-carrageenan has the ρ value around 1.5, and its conformation could be specified as a random coil[3,14]. However, the ρ value around 1.5 does not necessarily correspond to the chain flexibility in the case of the carrageenan family, since the B values (the flexibility parameter) were found to be small in the range of 0.05 to 0.12 for all cases, indicating the semi-rigid nature of carrageenan chains. The SAXS also confirms the results of the B values, where a good linearity was observed in the cross-sectional Guinier plots for respective carrageenans (see the discussion below). Thus the ρ value reflects an overall conformation, but the local conformation of the carrageenan family is still characterized as a stiff rod. Large ρ value seems a prerequisite for gelation of the carrageenan family since κ-, ι- and θ-carrageenan form gel but not λ- and β-carrageenan which possess relatively small ρ values. Although τ- and ρ-carrageenans possessing the ρ value around 1.7 form no gel in 0.1 M KCl aqueous solution, τ-carrageenan exhibits a tendency of gelation in higher salt concentration. The lack of sulfate groups on C2 of (1→3)-linked β-D-galactose seems to promote gelation. The details of gelation mechanism will be discussed in the succeeding paper.

The results of SAXS at 5°C and 60°C were analyzed conventionally by the Guinier approximation for cross-section[16]. Here the scattered intensity is approximately expressed in terms of the cross-sectional radius of gyration R_{gc} as

$$q \cdot I(q) \approx \exp(-R_{gc}^2 q^2 / 2) \tag{2}$$

Here q denotes the magnitude of the scattering vector given by

$$q = (4\pi / \lambda) \sin(\theta / 2) \tag{3}$$

with λ and θ being the wavelength of the incident beam and the scattering angle, respectively.

The SAXS results are summarized in Table 3. All Guinier plots for cross-section ($\ln qI(q)$ vs. q^2, see Equation (2)) yielded a good straight line, and the cross-sectional radius of gyration R_{gc} was estimated according to Equation (2). That is, a rod-like structure seems to specify the local conformation of the carrageenan. The cross-sectional radius of gyration of 0.50 nm corresponds well to a single (or double) helix conformation of poly-((1→3)-linked β-D-galactose and (1→4)-linked α-D-3,6-anhydrous-galactose. As the ρ value is around 1.7, an overall chain seems to assume a random coil conformation in the case of τ- and ρ-carrageenans. That is, τ- and ρ-carrageenans are composed of the fragments of rigid rods which are linked to constitute a whole chain. At I = 0.1, the influence of the charge density still remains on the chain stiffness as τ-carrageenan possessing less sulfate groups has a larger B value than ρ-carrageenan possessing more sulfate groups. Here τ-carrageenan was found to undergo gelation in higher polymer and salt concentration, though gel is weak and easily breaks to flow. ρ-carrageenan forms no gel under any circumstances. Comparing the chemical structure of τ- and ρ-carrageenans, the sulfate groups on C2 of (1→3)-linked β-D-galactose seems to prevent gelation.

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