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Structure and Gelling Properties of Carrageenan Family Studied by Scattering Techniques

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A series of regio-selectively sulfated carrageenans was prepared by sulfation and subsequent desulfation at specific positions. Prepared carrageenans were molecularly characterized by means of static/dynamic light scattering and small-angle X-ray scattering. The results have indicated that the shape of overall carrageenan chains depends on the position of sulfate groups as represented by a rod-like molecule, a random coil or a compact particle-like molecule, although the local chain behaves rod-like in any carrageenan. When a carrageenan has a rod-like shape as a whole, its aqueous solution forms gel at lower temperatures. The sulfate groups on C2 of (1→3)-linked β-D-galactose break a helical conformation, while the sulfate groups on C4 of (1→3)-linked β-D-galactose stabilize it as confirmed by the model energy calculation for hypothetical carrageenan single or double helices. The helical conformation of a certain length seems to be prerequisite for gelation.

Key Words: Carrageenan, Structure, Gelation, Light scattering, Small angle X-ray scattering.

INTRODUCTION

Carrageenans address generally the sulfated polysaccharides extracted from the cell wall of red seaweeds. Carrageenans are basically constituted of disaccharide repeating units of alternating (1→3)-linked β-D-galactose and (1→4)-linked α-D-galactose. Here the (1→3)-linked β-D-galactose units are partially sulfated at the positions of C2 and/or C4, and the (1→4)-linked α-D-galactose units are occasionally 3,6-anhydride by the sulfation at C2[1]. Natural carrageenans are classified conventionally as kappa (κ-), iota (ι-) and lambda (λ-) according to the number and position of sulfate groups in the repeating units[2] as shown in Fig. 1. Here alpha (α-), beta (β-), theta (θ-), tau (τ-), and rho (ρ-) carrageenans are also classified, but

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are rarely or not found in nature. Naturally available κ-, ι- and λ-carrageenans are widely applied as a viscosity intensifier in the food and cosmetic industry[3].

Thermoreversible gelation takes place in the aqueous solutions of κ-, ι- and θ-carrageenans with or without the presence of salt, but not in the aqueous solution of λ-carrageenan[4]. This different gelling behaviour is thought to be due to the number/position of sulfated groups and/or the conformational restriction by anhydrous residues. Here ideal κ- or ι-carrageenan should possess one or two sulfate groups per repeating unit at C4 of (1→3)-linked β-D-galactose or C2 of (1→4)-linked α-D-galactose and C4 of (1→3)-linked β-D-galactose, respectively, as seen from Fig. 1. Ideal θ-carrageenan possesses two sulfate groups at respective C2 positions of (1→3)-linked β-D-galactose and (1→4)-linked α-D-galactose, while λ-carrageenan contains three sulfate groups at C2 of (1→3)-linked β-D-galactose and at C2/C6 of (1→4)-linked α-D-galactose.

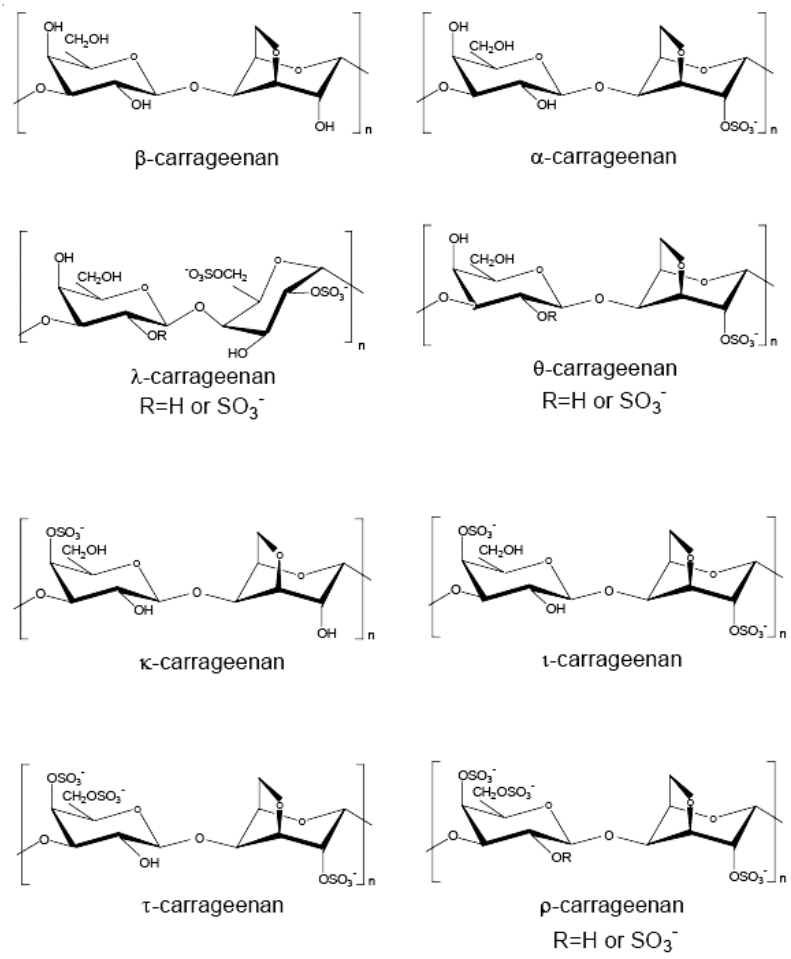


Fig. 1. Chemical structure of carrageenan family

Although the coil-to-helix transition and the subsequent lateral association of helices are thought to cause gelation, the argument still remains whether it forms single or double helices[5-7]. Apart from the argument whether the carrageenan helix is single or double, the mode of a specific intermolecular interaction is not well understood. In the preceding paper[4,8], a series of carrageenans as prepared by desulfation, where the number and position of sulfate groups on (1→3)-linked β-D-galactose and (1→4)-linked α-D-galactose vary as shown in Fig. 1, and their dilute solution properties were examined by combining light scattering and small-angle X-ray scattering. The role of sulfate groups and (1→4)-linked anhydrous α-D-galactose on the conformational characteristics as discussed in some extent in order to explain the variation of an overall chain conformation with carrageenan species of similar local chain stiffness.

We aim to clarify in this paper the mode of intermolecular interaction by specifying the number and/or position of sulfate groups on (1→3)-linked β-D-galactose and (1→4)-linked α-D-galactose. A specific interaction leads to gelation in the aqueous solution of the carrageenan family. The small-angle X-ray scattering and NMR are applied to analyze the microstructure and the exact position of sulfate groups in the ordered domain formed in the process of gelation. The static and dynamic light scattering is applied to elucidate the conformational characteristics of carrageenan chains in sol state. By combining information on local and overall structures of a carrageenan chain and its aggregated domain, we focus our discussion on the position of the specific groups in the molecular assembly. Since gelation is related to the formation of ordered domain, the role of sulfate groups is discussed from the molecular model in the formation of ordered domains.

EXPERIMENTAL

Preparation and characterization of the carrageenan family: ι-, κ- and λ-carrageenans of the carrageenan family were purchased from Sigma and treated with ion-exchange resins and NaOH solution. Resulting Na-form carrageenan samples were used for molecular characterization. β-Carrageenan was prepared from κ-carrageenan with silylating reagent[4,9]. θ-Carrageenan was prepared by alkaline treatment of λ-carrageenan[4,10,11]. Novel tri- and tetra-sulfated carrageenans were prepared by sulfation at specific positions[8,12,13]. Since there is no systematic way to code the carrageenans in a conventional terminology, those carrageenans were coded briefly as τ-(tri-sulfated) and ρ-carrageenan (tetra-sulfated), respectively, for citing convenience[8,14]. Although α-carrageenan cannot be prepared by the present method[15], a considerable part of θ-carrageenan prepared as above misses the sulfate group on C2 of (1→3)-linked β-D-galactose and is equivalent to α-carrageenan. Thus the model calculation includes *f*Ñ-carrageenan in later discussion.

Each carrageenan was purified by dialysis and subsequent percolation of respective aqueous solutions (C_p = 2 wt %) through an ion exchange column was performed. The number of sulfate groups per repeating disaccharide unit was determined by

conductometric titration using NaOH aqueous solution. The sulfate groups of carrageenan samples were converted into H-form by passing through a cation-exchange resin before titration.

The chemical structures of carrageenan samples were confirmed by ^{13}C NMR. The detail procedure and the assignment of chemical shifts will be found in the preceding papers[4,8,16]. 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 chromatograph[4,17,18]. The weight-average molecular weights of the carrageenan samples were estimated by static light scattering observed from the 0.1 M NaCl aqueous solution with the ALV Spectrometer 5000. A He-Ne laser (λ_0 = 633 nm) was employed for a light source and the optical constant was calibrated by toluene as a primary standard. The chemical and molecular characteristics of the obtained carrageenans were summarized in Table-1. The detail procedure of the sample preparation and characterization is found in the preceding papers[4,8].

TABLE-1
CHEMICAL AND MOLECULAR CHARACTERISTICS OF CARRAGEENAN FAMILY

Carrageenan type	Gal-Gal/Gal-Angal (molar ratio)	Sulfate content (mol H^+/mol disaccharide)	dn/dc (mL/g)	$M_w \times 10^{-3}$ (g/mol)	$A_2 \times 10^3$ (mol mL/g)	R_g (nm)	R_H (nm)	ρ (=R_g/R_H)
β	1:12	0.17	0.140	197	-0.71	74.5	47.2	1.58
β*	1:12	0.17	0.135	147	2.11	60.7	43.8	1.39
θ	1:4	1.37	0.116	408	0.44	73.5	30.1	2.44
λ	1:0.33	2.20	0.122	2399	0.13	109.0	118.0	0.92
κ	1:15	0.92	0.126	371	1.10	66.8	29.7	2.25
ι	1:18	1.65	0.124	543	0.07	73.3	32.7	2.24
τ	1:18	2.50	0.117	492	0.85	63.1	38.5	1.64
ρ	1:18	3.11	0.115	390	-0.40	54.4	31.3	1.74

*No salt is added; R_g and R_H denote the radius of gyration and the hydrodynamic radius of gyration, respectively.

Small-angle X-ray scattering (SAXS): The SAXS was observed with the small-angle X-ray scattering equipment for solution (SAXES) installed at BL-10C section of the Photon Factory, Tsukuba, Japan, from the aqueous solutions of κ-, ι-, λ-, β-θ-, τ-, and ρ-carrageenan with or without salt at 60 ºC and 5 ºC. An incident X-ray beam was monochromatized to λ = 0.149 nm and focused to the position of the detector with a bent focusing mirror. The scattered X-ray was detected by a one-dimensional position sensitive proportional counter (PSPC) set at the distance of about 1 m from the cell holder. The solutions were injected in a flat cell of 0.2 cm path-length made of glass with quartz windows (20 μm thick), which was placed in the cell holder kept at a predetermined temperature at least 10 min prior to the SAXS measurements. The SAXS intensities were accumulated for total 600 s in order to ensure enough statistical accuracy without degrading the samples by

X-ray. The observed SAXS intensities were corrected with respect to the variation of the incident X-ray flux by monitoring an ion chamber placed in front of the cell holder. The excess scattering intensities I(q) ($q = (4\pi/\lambda)\sin(\theta/2)$ with λ and θ being the wavelength of an incident light and the scattering angle, respectively) were calculated by subtracting the scattered intensities of the solvent from those of the respective solutions. The results were analyzed conventionally by plotting $q^2I(q)$ against q (the Kratky plots) and lnqI(q) against q^2 (the cross-sectional Guinier plots). The molecular models were built by computer simulation to yield the observed scattering profile[19].

Gelling behaviour: κ-, ι- and θ-carrageenan aqueous solutions are known to form thermoreversible gel with the presence of metal counter ions[4]. On the other hand, λ-carrageenan aqueous solution does not form gel under any condition. Gel formation at 5 and 60 ºC was checked by the naked eye for the alkali- and alkaline-earth-salt aqueous solutions of the carrageenan. The results are summarized in Table-2, together with the cross-sectional radius of gyration estimated from the SAXS measurements[4,8].

Although several solutions do not show gelation to the naked eye, the SAXS profiles in the Kratky plots exhibit a sharp upturn at $q \rightarrow 0$ (Fig. 2) considered as a symptom of gelation according to the classic theory of gelation[20]. Those are noted as gel in the bracket in Table-2.

RESULTS AND DISCUSSION

As seen from Table-1, not all the carrageenans exhibit a rod-like nature when the ρ (the ratio of the radius of gyration to the hydrodynamic radius) value is chosen for its criterion. However, the cross-sectional Guinier plots yield a region of a straight line as seen from Fig. 3 and confirm the local chain stiffness of the carrageenan family. The cross-sectional radius of gyration in respective solutions at 60 ºC was found to increase in proportion to the number of sulfate groups (Fig. 4). This tendency can be visualized in respective molecular models after the energy optimization as shown in Fig. 5 for a single coil. When compared with the gelation behaviour, the rod-like nature in the local chain with a certain length may be a prerequisite to form a strong gel. The sulfate groups on C4 of (1→3)-linked β-D-galactose and on C2 of anhydrous (1→4)-linked α-D-galactose seem to enforce a chain stiffness by stabilizing a helical structure, but that on C2 of (1→3)-linked β-D-galactose functions as a helix breaker[21]. The sulfate on C2 of (1→3)-linked β-D-galactose may form a hydrogen bond with the hydroxyl group on C3 of (1→4)-linked α-D-galactose[22] to twist the conformation at this position. When no sulfate group attaches to C4 of (1→3)-linked β-D-galactose and to C2 of anhydrous (1→4)-linked α-D-galactose, the carrageenan chain is rather flexible as seen from the ρ value (1.58) of β-carrageenan. β-Carrageenan forms gel only in high polymer and salt concentrations. Since no strong interaction between chains is present, its gel is weak and breaks easily to flow.

TABLE-2
CROSS-SECTIONAL RADIUS OF GYRATION AND GELLING BEHAVIOR OF CARRAGEENAN FAMILY gel/sol WAS JUDGED FROM THE FLOW BEHAVIOUR OF THE SOLUTION IN THE TILTED BOTTLE BY EYE INSPECTION

Carrageenan type (concentration)	Added salt	R_{gc} (nm)		gel or sol	
		5 ºC	60 ºC	5 ºC	60 ºC
β (C_P = 1.5 %)	0.1 M NaCl	–*	0.28	sol (gel)†	sol
	0.1 M KCl	–*	0.32	sol (gel)†	sol
	0.1 M $CaCl_2$	–*	0.28	sol (gel)†	sol
β (C_P = 3.0 %)	0.1 M NaCl	–*	0.31	sol (gel)†	sol
	0.1 M KCl	–*	0.32	sol (gel)†	sol
	0.1 M $CaCl_2$	–*	0.34	sol (gel)†	sol
θ (C_P = 1.5 %)	0.1 M NaCl	0.45	0.40	sol	sol
	0.1 M KCl	1.15	0.43	gel	sol
	0.1 M $CaCl_2$	0.51	0.47	sol	sol
λ (C_P = 1.5 %)	0.1 M NaCl	0.43	0.43	sol	sol
	0.1 M KCl	0.44	0.44	sol	sol
	0.1 M $CaCl_2$	0.49	0.49	sol	sol
κ (C_P = 1.5 %)	0.1 M NaCl	0.39	0.32	sol	sol
	0.1 M KCl	0.85	0.35	gel	sol
	0.1 M $CaCl_2$	0.38	–*	gel	sol
ι (C_P = 1.5 %)	0.1 M NaCl	0.51	0.41	gel	sol
	0.1 M KCl	0.66	0.46	gel	sol
	0.1 M $CaCl_2$	0.55	0.54	gel	sol
τ (C_P = 1.5 %)	0.1 M NaCl	0.50	0.48	sol	sol
	0.1 M KCl	0.48	0.47	sol	sol
	0.5 M KCl	0.51	0.47	sol (gel)†	sol
	0.1 M $CaCl_2$	0.55	0.54	sol	sol
τ (C_P = 3.0 %)	0.5 M KCl	0.60	0.47	gel	sol
ρ (C_P = 1.5 %)	0.1 M NaCl	0.50	0.49	sol	sol
	0.1 M KCl	0.50	0.49	sol	sol
	0.1 M $CaCl_2$	0.56	0.56	sol	sol
ρ (C_P = 3.0 %)	0.5 M KCl	–**	0.49	sol	sol

*No apparent linear region was observed in the cross-sectional Guinier plots.
**The sample was destroyed by X-ray.
†(gel) denotes the judgment from the SAXS profile.

ρ-Carrageenan carries a sulfate group on C2 and C6 of (1→3)-linked β-D-galactose units and assumes a rather compact conformation. ρ-Carrageenan forms no gel even in high polymer and salt concentrations. However, τ-carrageenan having a sulfate group on C6 but not on C2 of (1→3)-linked β-D-galactose, forms gel at high salt and polymer concentrations. The results indicate that the sulfate groups on C2 of (1→3)-linked β-D-galactose destabilize the helical structure of the chain. Although the effect of the sulfate groups on C6 of (1→3)-linked β-D-galactose is less remarkable on the carrageenan conformation, it also destabilizes the helical structure as known by comparing the gelling behaviour of τ-carrageenan with ι-carrageenan. τ-carrageenan has a lower ρ value (a ratio of the radius of gyration to

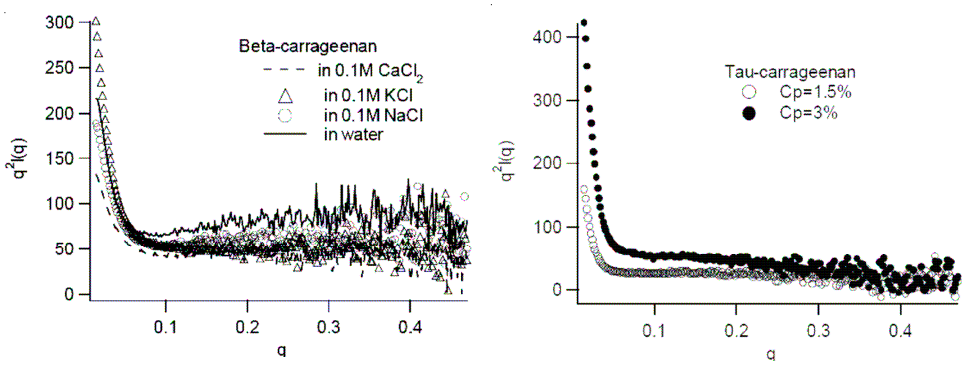


Fig. 2. An example of small-angle X-ray scattering. The Kratky plots for 1.5 % β-carrageenan aqueous solution with/without salt (indicated in the figure) at 5 ºC (top) and 1.5/3.0 % τ-carrageenan in 0.5 M KCl aqueous solution at 5 ºC (bottom)

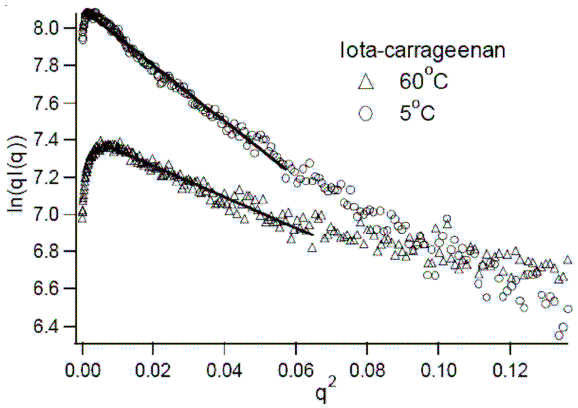


Fig. 3. Guinier plot for cross-section from 1.5 % ι-carrageenan in 0.1 M NaCl aqueous solution at 5 and 60 ºC

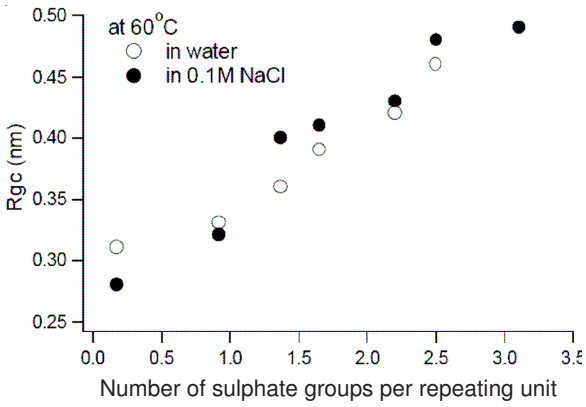
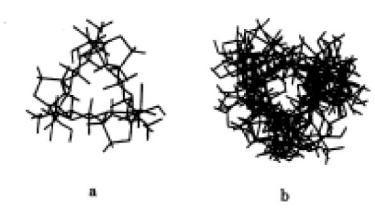
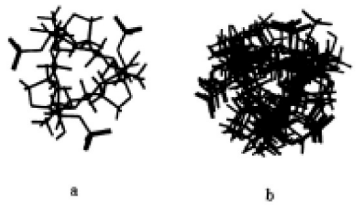


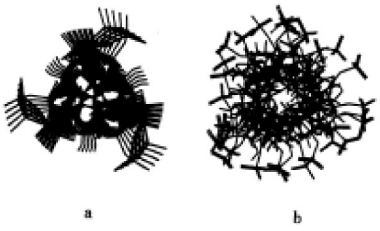
Fig. 4. Cross-sectional radius gyration as a function of the number of sulfate groups per repeating unit



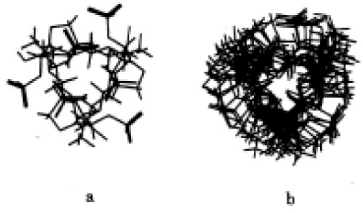
β-carrageenan



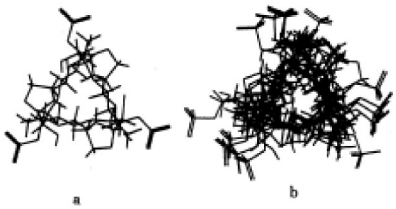
α-carrageenan



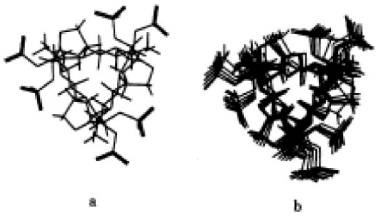
λ-carrageenan



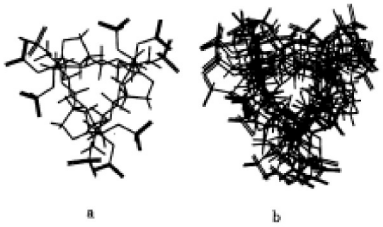
θ-carrageenan



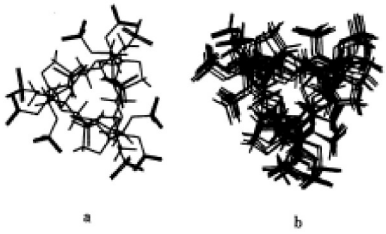
κ-carrageenan



ι-carrageenan



τ-carrageenan



ρ-carrageenan

Figure continued

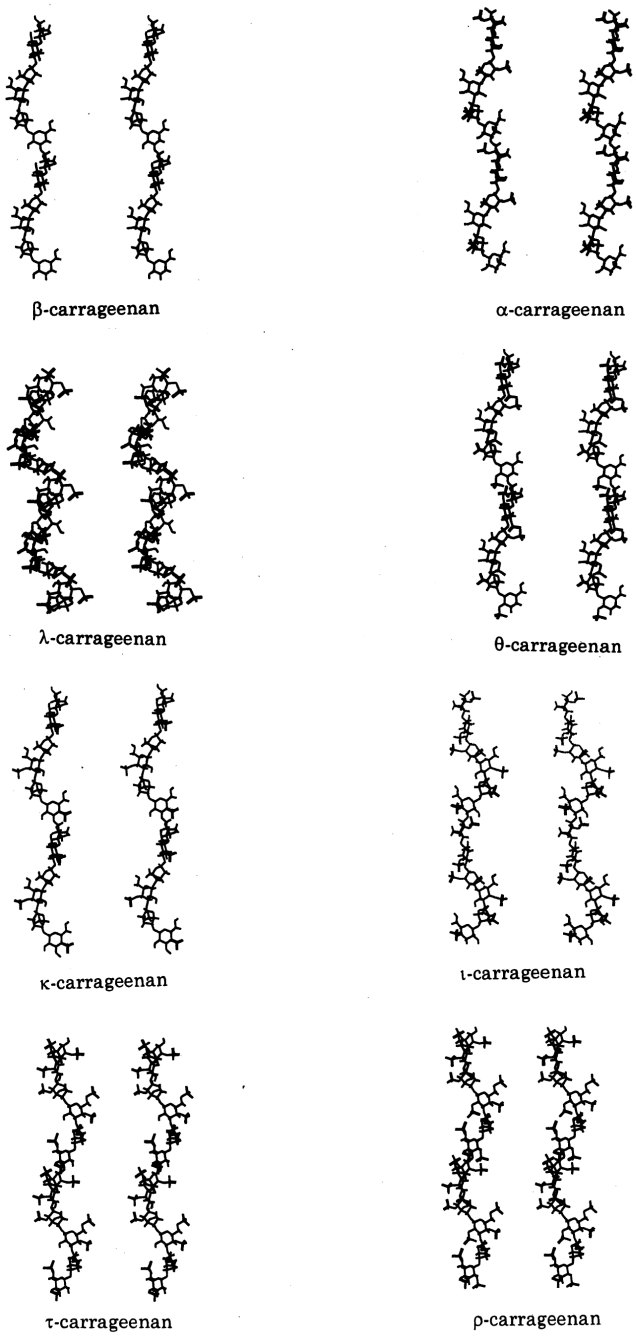


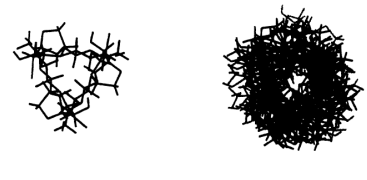
Fig. 5. Molecular model of a single helix of the carrageenan family. Each top view shows the original (a) and energy-optimized (b) conformation of respective carrageenan (indicated in the figure) and the side view (a stereo figure) corresponds to original conformation composed of 30 residues

the hydrodynamic radius) than ι-carrageenan, confirming that an overall rigid rod-like conformation is broken more frequently.

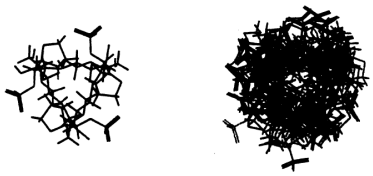
In its ideal structure, θ-carrageenan carries a sulfate group on C2 of (1→3)-linked β-D-galactose unit but not on C6. However, total sulfate content was evaluated as 1.37 per disaccharide unit for θ-carrageenan. Considering the anhydrous (1→4)-linked α-D-galactose unit carries one sulfate group and its fraction is 0.8 estimated from the GLC results, the fraction of the sulfated groups on C2 of (1→3)-linked β-D-galactose is calculated as 0.17 for θ-carrageenan from Table-1 by assuming one sulfate group attached to C2 of anhydrous (1→4)-linked α-D-galactose. θ-Carrageenan prepared here is more like α-carrageenan in definition. Similarly the fraction of the sulfated groups on C2 of (1→3)-linked β-D-galactose is calculated as 0.45 for λ-carrageenan. A small amount of the sulfate groups on C2 of (1→3)-linked β-D-galactose may not disturb the gelation of θ-carrageenan too much and θ-carrageenan assumes a rod-like structure enforced by the sulfate group on C2 of anhydrous (1→4)-linked α-D-galactose as indicated a high ρ value (2.44, Table-1) despite of the presence of sulfated C6 in (1→4)-linked α-D-galactose units.

The sulfate group on C6 of (1→4)-linked α-D-galactose unit breaks the anhydrous structure, leading to the large conformational change. Here, as seen from the example of λ- and θ-carrageenan, the conformational change seems to require both non-anhydrous (1→4)-linked α-D-galactose units and a sulfate group on C2 of (1→3)-linked β-D-galactose units. λ-Carrageenan has an extremely small value of ρ (=0.92) and does not form gel under any circumstances. On the other hand, θ-carrageenan has a large ρ value (2.44) assigned to a rod-like structure.

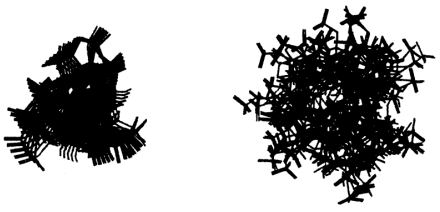
To understand the mode of interaction better, we construct the molecular model of the carrageenan family, and observe the relative position of sulfate groups. The molecular models of respective carrageenans were constructed on the basis of the available crystallographic data of ι-carrageenan[23] and λ-carrageenan[24]. Either a double helix or a single helix composed of 30 residues per helix was constructed at first from the data of ι-carrageenan or λ-carrageenan, respectively. The double helices for α-, β-, κ-, θ-, τ- and ρ-carrageenan were made from that of ι-carrageenan by adding and/or eliminating sulfate groups to/from appropriate positions. α-, β-, κ-, θ-, τ- and ρ-carrageenan single helices denote one side of respective double helices. The double helix for λ-carrageenan was artificially constructed by coupling a pair of λ-carrageenan single helices. The total energy was calculated from those molecular models directly and then optimized by Discover Molecular Simulation Program (Molecular Simulation Inc.). The results are summarized in Figs. 5 and 6 (Table-3). Here α-carrageenan (which corresponds to θ-carrageenan without sulfate groups on C2 of (1→3)-linked β-D-galactose) is included in the model, since only a small amount of sulfate groups were found to be attached on C2 of (1→3)-linked β-D-galactose in prepared θ-carrageenan. As seen from Table-3, all carrageenans forming gel have relatively low total and non-bond potential energy at the initial (either



β-carrageenan

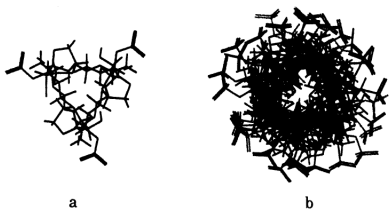


α-carrageenan



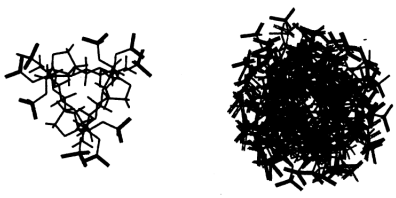
λ-carrageenan

θ-carrageenan

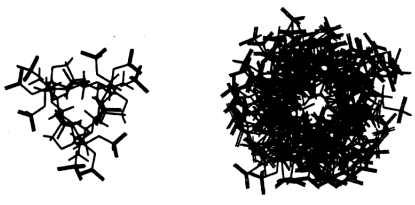


κ-carrageenan

ι-carrageenan



τ-carrageenan



ρ-carrageenan

Figure continued

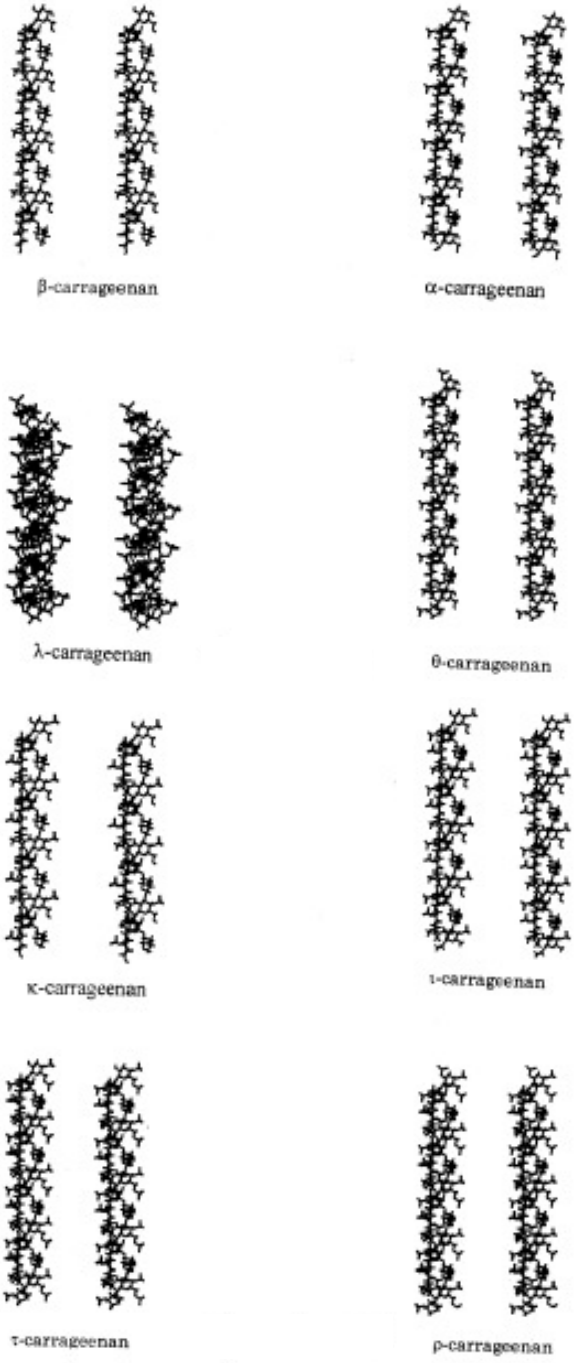


Fig. 6. Molecular model of a double helix of the carrageenan family. Each top view shows the original (a) and energy-optimized (b) conformation of respective carrageenan (indicated in the figure), and the side view (a stereo figure) corresponds to the original conformation composed of 30 residues

single or double) helix stage except for θ-carrageenan. Thus, those carrageenans which are capable of maintaining the helical conformation with a certain length enough to associate with each other to form the crosslinking domain. Here we use both a single and a double helix for the model calculation, because there is still the argument whether the ordered domain is composed of a single or a double helix. As far as the present analysis will concern, a similar tendency was observed with either a single or a double helix, and there is no fundamental difference in the discussion regardless of the helix model employed here. After the energy optimization, the helical conformation becomes skewed (Figs. 5 and 6). We recognize more skewed structure in less gel-forming carrageenan, although no quantitative evaluation of conformational skewness and gelling potential can be made at present. Sulfate groups on C2 of (1→3)-linked β-D-galactose situate inside of the helical conformation and increase the potential energy considerably. Thus the helical conformation of the carrageenans with sulfate groups on C2 of (1→3)-linked β-D-galactose is hardly maintained in aqueous solution and no further association of helices will take place. Sulfate groups on C4 of (1→3)-linked β-D-galactose stick out from the helix and may promote the association of helices by ion bridging. The results of helical model simulation confirm the above discussion.

TABLE-3
POTENTIAL ENERGY OF A SINGLE AND DOUBLE HELIX COMPOSED OF 30 RESIDUES OF THE CARRAGEENAN FAMILY. THE VALUE IN THE BLANKET CORRESPONDS TO THE DOUBLE HELIX. THE INITIAL VALUE CORRESPONDS TO THE POTENTIAL ENERGY OF THE ORIGINAL HELIX, AND THE FINAL VALUE TO THE OPTIMIZED POTENTIAL ENERGY

Carrageenan	Number of residue	Energy (kJ mol^{-1})			
		Total		Non-bond	
		Initial	Final	Initial	Final
β	30	5.90×10^3 (1.13×10^4)	1.33×10^2 (-5.36×10^3)	5.69×10^3 (1.29×10^4)	2.43×10^3 (-1.92×10^3)
α	30	5.02×10^3 (4.44×10^4)	2.17×10^2 (-4.85×10^3)	4.18×10^3 (4.18×10^4)	2.00×10^3 (-2.90×10^3)
θ	30	5.82×10^5 (2.04×10^6)	4.69×10^2 (-5.02×10^3)	5.69×10^5 (2.02×10^6)	(9.83×10^2) (-5.02×10^3)
λ	30	8.62×10^8 (4.44×10^{11})	-1.38×10^3 (1.08×10^4)	8.62×10^8 (4.44×10^{11})	-2.96×10^3 (-3.42×10^3)
κ	30	5.94×10^3 (1.23×10^4)	3.27×10^2 (-6.36×10^3)	5.02×10^3 (1.18×10^4)	2.02×10^3 (-4.14×10^2)
ι	30	4.27×10^3 (4.15×10^4)	1.53×10^2 (-5.82×10^3)	3.37×10^3 (3.94×10^4)	1.26×10^3 (-4.60×10^3)
τ	30	1.11×10^4 (5.52×10^4)	3.29×10^2 (-2.78×10^3)	3.27×10^3 (3.90×10^4)	6.65×10^2 (-3.23×10^3)
ρ	30	5.86×10^5 (2.05×10^6)	2.27×10^2 (-2.91×10^3)	5.73×10^5 (2.02×10^6)	-1.76×10^2 (-6.07×10^3)

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