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CARRAGEENAN SYSTEMS FROM TETRASPORIC AND CYSTOCARPIC STAGES OF *GIGARTINA SKOTTSBERGII*

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Key Word Index—*Gigartina skottsbergii*; Rhodophyta; Gigartinaceae; seaweed; nuclear phases; carrageenans.

Abstract—Cystocarpic and tetrasporangial plants of *Gigartina skottsbergii* produced different systems of carrageenans. These carrageenans were fractionated with potassium chloride and analysed. The ranges of precipitation, the molar ratios Gal:3,6-AnGal:sulphate and the IR spectra of the fractions indicated that the cystocarpic system was composed of similar amounts of gelling and soluble carrageenans of the κ/ι- and μ/ν-type, respectively. The tetrasporic system was composed of a major fraction of the λ- type precipitated at 0.60–1.00 M KCl and two minor fractions, one of the λ- type precipitated at 1.10–1.30 M KCl and the other soluble in 2.00 M KCl with a λ- like IR spectrum but μ/ν- like analytical data (ω-carrageenan). The major cystocarpic and tetrasporic carrageenan fractions were submitted to alkaline treatment and further fractionation with potassium chloride. The modified cystocarpic polysaccharide fractions gave products comprising mainly gelling carrageenans while the modified tetrasporic polysaccharide fractions yielded soluble carrageenans in agreement with previously assigned structures.

INTRODUCTION

Carrageenans are different in karyologically different generations as shown in *Chondrus* [1, 2], *Gigartina* [1, 3–5] and *Iridaea* [3, 4, 6] all of which belong to the family Gigartinaceae in the red algae. In this family 'κ'- and 'λ'- carrageenans are produced by haploid (cystocarpic) and diploid (tetrasporic) individuals, respectively. On the other hand, in the other related families of the order Gigartinales, Hypneaceae [7], Furcellariaceae [8] and Solieriaceae [9] both nuclear phases yield the same system of polysaccharides. An exception to this rule has been reported by Ayal and Matsuhiro [10] who found that the tetrasporic and cystocarpic phases of *Chondrus canaliculatus* produced similar mixtures of hybrid polymers.

In most of the previous work carrageenan systems were not studied because the raw polysaccharides were not fractionated or the 'κ'- and 'λ'- carrageenans were not fully characterized. We wish to report the carrageenan systems synthesized by the nuclear phases of *G. skottsbergii*, its fractionation and the characterization of each fraction by potassium chloride precipitation, the molar ratio Gal:3,6-AnGal:sulphate and the IR spectrum. The major fractions were submitted to alkaline treatment and the products obtained were fractionated. Completely different patterns were obtained for the cystocarpic and the tetrasporic carrageenans which show the complexity of the systems biosynthesized by each phase.

RESULTS

Cystocarpic and tetrasporangial plants of *G. skottsbergii* were sequentially extracted with water at room temperature. Yields, analyses, and optical rotations of the crude carrageenans are given in Tables 1 and 2. Details of the fractionation of the cystocarpic carrageenan 1 C in solutions of increasing potassium chloride concentration are given in Table 1. According to the ranges of precipitation and the molar ratios of the fractions, the crude carrageenan 1 C is composed by similar amounts of gelling and soluble compounds of the κ/ι- and μ/ν-type, respectively. The IR spectra show the expected bands at 930 cm^{-1}, typical of the presence of 3,6-anhydrogalactose, and 845–855 cm^{-1}, due to secondary axial sulphate groups [11]. Fractionation of the carrageenan 1 T with potassium chloride indicated the lack of κ/ι-like carrageenans in the crude sample and the presence of a major fraction precipitated between 0.60–1.00 M KCl (88.5% of recovered) and two minor fractions, one precipitated between 1.10–1.30 M (5.9% of recovered) and the other soluble in 2.00 M KCl (5.5% of recovered, 1 T_7) (Table 2). The major fraction was arbitrarily subfractionated into four subfractions (1 T_1, 1 T_2, 1 T_3 and 1 T_4) (Table 2), the higher yield of precipitation was found in the range 0.70–0.80 M KCl (45.3% of recovered, 1 T_2). Analytical data are similar for the four subfractions with the exception of C-6 sulphate which decreases with increasing solubility (Table 2); the molar ratio Gal:3,6-AnGal:sulphate are typical of λ-carrageenan. The minor product precipitated between 1.10 and 1.30 M KCl was also arbitrarily subfractionated giving two subfractions with the same λ-like analytical data (1 T_5 and 1 T_6) (Table 2). The IR spectra of fractions 1 T_1–1 T_3 and 1 T_6 show no bands at 930 cm^{-1} and the main absorption is centred at

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Table 1. Yields, analyses and optical rotations of the cystocarpic carrageenan and of the fractions isolated from it by precipitation with potassium chloride

Fraction	Range of precipitation (M KCl)	Yield* (%)	C-6 sulphate (as SO_3K) (%)	Gal : 3,6-AnGal : sulphate molar ratio	$[\alpha]_D$ (°)
1 C†	—	49.1	—	1.00 : 0.77 : 1.25	+61.6
2 C†	—	13.9	—	1.00 : 0.63 : 0.98	+61.7
1 C_1	0.30–0.31	33.0 (42.6)	7.9	1.00 : 0.64 : 1.32	+66.5
1 C_2	0.40–0.42	2.7 (3.5)	11.8	1.00 : 0.62 : 1.20	+56.1
1 C_3	2.00‡	41.7 (53.9)	34.1	1.00 : 0.37 : 1.14	+55.1

*Yields are given for 100 g of seaweed for fraction 1 C and 2 C and for 100 g of carrageenan (in parentheses, per cent of total recovered) for the other fractions.

†Fractions 1C and 2C correspond to the unfractionated carrageenans from the first and second extractions, respectively. A third extraction did not produce any polysaccharide.

‡Soluble in 2.0 M KCl.

Table 2. Yields, analyses and optical rotations of the tetrasporic carrageenan and of the fractions isolated from it by precipitation with potassium chloride

Fraction	Range of precipitation (M KCl)	Yield* (%)	C-6 sulphate (as SO_3K) (%)	Gal : 3,6-AnGal : sulphate molar ratio	$[\alpha]_D$ (°)
1 T†	—	43.2	—	1.00 : 0.06 : 1.77	+75.0
2 T†	—	7.6	—	1.00 : 0.03 : 1.13	+128.0
1 T_1	0.60–0.70	13.0 (15.8)	16.2	1.00 : 0.03 : 1.12	+116.4
1 T_2	0.70–0.80	37.3 (45.4)	9.8	1.00 : 0.03 : 1.20	+70.8
1 T_3	0.80–0.90	13.9 (16.9)	1.4	1.00 : 0.03 : 1.16	+76.3
1 T_4	0.90–1.00	8.6 (10.4)	1.4	1.00 : 0.03 : 1.20	+84.0
1 T_5	1.10–1.15	3.1 (3.8)	n.d.	1.00 : 0.05 : 1.15	+96.1
1 T_6	1.15–1.30	1.7 (2.1)	n.d.	1.00 : 0.05 : 1.07	+119.8
1 T_7	2.00‡	4.5 (5.5)	n.d.	1.00 : 0.38 : 0.89	+3.7

*Yields are given for 100 g of seaweed for fraction 1 T and 2 T and for 100 g of carrageenan (in parentheses, per cent of total recovered) for the other fractions.

†Fractions 1 T and 2 T correspond to the unfractionated carrageenans from the first and second extractions, respectively. A third extraction did not produce any polisaccharide.

‡Soluble in 2.0 M KCl.

830 cm^{-1} indicating the prevalence of secondary equatorial sulphate groups [11]. The soluble fraction (1 T_7) has μ/ν-like analytical data (Table 2) but the IR spectrum shows a band at 830 cm^{-1} and two small shoulders at 925 and 845 cm^{-1}. The cystocarpic carrageenan fractions (1 C_1, 1 C_2 and 1 C_3) were submitted to alkaline treatment (1 C_1T, 1 C_2T and 1 C_3T) (Table 3); the two major fractions (1 C_1T and 1 C_3T) were further fractionated with potassium chloride (Table 3). 1 C_1T contained 97.1% of gelling carrageenans (κ/ι-carrageenans) precipitated between 0.05–0.10 M KCl (81.7% of recovered, 1 C_1T_1) and 0.25–0.29 M KCl (15.4% of recovered, 1 C_1T_2) and a small amount of a product soluble in 2.00 M KCl (2.9% of recovered, 1 C_1T_3). The alkali-treated 1 C_3T produced 89.8% of gelling carrageenans precipitated between 0.05–0.10 M KCl (77.9% of recovered, 1 C_3T_1) and 0.10–0.15 M KCl (11.9% of recovered, 1 C_3T_2), and a small amount of a product soluble in 2.00 M KCl (6.3% of recovered, 1 C_3T_4). The IR spectra of the modified cystocarpic carrageenan fractions and of the corresponding subfractions obtained by fractionation with potassium chloride are similar with major absorptions at 835–860 cm^{-1}, 930 cm^{-1} and a small peak at 805 cm^{-1} which indicates the presence of 3,6-anhydrogalactose 2-sulphate units [12]. It is noteworthy that both alkali-treated derivatives 1 C_1T_3 and 1 C_3T_4 have analytical data and IR spectra of the κ-family [13], in spite of being soluble in potassium chloride solutions.

The major gelling fraction produced by the cystocarpic plant (1 C_1, Table 1) is composed by two κ/ι-like carrageenans (see 1 C_1T_1 and 1 C_1T_2, Table 3) and a small amount of a soluble μ/ν-like carrageenan (see C_1T_3, Table 3). On the other hand, the soluble fraction (1 C_3, Table 1) comprises mainly a μ/ν-like carrageenan (see 1 C_3T_1 and 1 C_3T_2, Table 3) and minor amounts of a soluble carrageenan with characteristics of the κ-family (see 1 C_3T_4, Table 3). Nevertheless the major ι-fraction (1 C_1) was a mixture of different carrageenans, it precipitated in a narrow range of potassium chloride concentration (0.30–0.31 M, Table 1), suggesting, as previously reported [14, 15], that the molecules can co-precipitate or form mixed aggregates. It is noteworthy that some of

Table 3. Alkaline treatment of carrageenan fractions from the cystocarpic stage

Fraction	Range of precipitation (M KCl)	Yield* (%)	Gal : 3,6-AnGal : sulphate molar ratio	$[\alpha]_D$ (°)
1 C_1T	—	86.7	1.00:0.72:1.36	+53.2
1 C_1T_1	0.05–0.10	72.8 (81.7)	1.00:0.95:1.77	+54.5
1 C_1T_2	0.25–0.29	13.7 (15.4)	1.00:0.72:1.31	+67.0
1 C_1T_3	2.00†	2.6 (2.9)	1.00:0.43:0.93	+7.8
1 C_2T	—	81.8	1.00:0.68:1.38	+53.6
1 C_3T	—	72.7	1.00:0.62:1.12	+48.3
1 C_3T_1	0.05–0.10	40.0 (77.9)	1.00:1.11:1.26	+59.9
1 C_3T_2	0.10–0.15	6.1 (11.9)	1.00:1.27:1.62	+56.9
1 C_3T_3	0.15–2.00‡	2.0 (3.9)	1.00:0.35:0.99	+31.2
1 C_3T_4	2.00†	3.2 (6.3)	1.00:1.11:1.65	+63.2

Yields, analyses and optical rotations of the modified fractions and of the subfractions isolated from modified fractions 1 C_1T and 1 C_3T by precipitation with potassium chloride.

*Yields from alkaline treatment for 1 C_1T, 1 C_2T, and 1 C_3T. Yields from fractionation for the other fractions (in parentheses, percent of total recovered).

†Soluble in 2.00 M KCl.

‡Diffuse precipitation.

Table 4. Alkaline treatment of carrageenan fractions 1 T_1, 1 T_2, and 1 T_3 of the tetrasporic stage

Fraction	Range of precipitation (M KCl)	Yield* (%)	Gal : 3,6-AnGal : sulphate molar ratio	$[\alpha]_D$ (°)
1 T_1T	—	77.3	1.00:0.99:1.82	n.d.
1 T_1T_1	2.00†	92.2 (71.3)	1.00:0.88:1.72	+30.3
1 T_2T	—	76.5	1.00:0.56:1.54	n.d.
1 T_2T_1	2.00†	97.3 (74.4)	1.00:0.77:1.62	+79.1
1 T_3T		50.5	1.00:0.11:0.19	n.d.
1 T_3T_1	2.00†	58.4 (29.5)	1.00:0.13:0.19	+151.1

Yields, analyses and optical rotations of the modified fractions and of the subfractions isolated from them by precipitation with potassium chloride.

*Yields from alkaline treatment for 1 T_1T, 1 T_2T, and 1 T_3T. Yields from fractionation for the other fractions (in parentheses, yields from alkaline treatment).

†Soluble in 2.00 M KCl.

the fractions obtained after the alkaline treatment of the original μ/ν-carrageenan (1 C_3, Table 1) show 3,6-anhydrogalactose : galactose molar ratios higher than unity.

Biosynthesis of carrageenans is carried out in three successive steps [16], namely: (a) the formation of the galactan backbone, (b) its selective sulphation and (c) conversion to the 3,6-anhydrogalactose units. The selective sulphation of the 3-linked residues establishes the difference between the final products γ-, μ-, and ν-carrageenans which are eventually transformed into β-, κ- and ι-carrageenans, respectively, on one hand, and λ- and ξ-carrageenans on the other. No sulphation or C-4 sulphation produces carrageenans of the κ-group, while sulphation on C-2 gives rise to carrageenans of the λ-group. Sulphation of C-6 produces new types of carrageenans (ω-carrageenans) [15, 17] whose precursors have not yet been found. Sulphation of the 4-linked residue at C-2 and/or C-6 occurs in carrageenans of these groups.

The major tetrasporic carrageenan fractions (1 T_1, 1 T_2, and 1 T_3) were submitted to alkaline treatment (1 T_1T, 1 T_2T, and 1 T_3T); further fractionation with potassium chloride yielded only non-gelling carrageenans (Table 4). It is noteworthy that 1 T_3 produced by alkaline treatment, a derivative with a low percentage of 3,6-anhydrogalactose, in agreement with the low amount of C-6 sulphate in the original product, but also with a low percentage of total sulphate, suggesting: (a) that some sulphate groups were split off without producing the anhydro derivative or (b) an heterogeneous distribution of these groups in the molecules with loss of the heavily sulphated ones during dialysis. This latter possibility is consistent with the low percentage recovery. The IR spectra show absorptions at 930, 820–830 and 805 cm^{-1}.

DISCUSSION

The characterization of different types of carrageenans present in a crude extract of a seaweed should be done not

only by complete analysis and determination of the molar ratio Gal:3,6-AnGal:sulphate but also by careful fractionation of the sample at different concentrations of potassium chloride, at least up to 2 M, and alkaline treatment of each of the fractions. This last step is necessary considering that carrageenans of different structural types can co-precipitate and/or form insoluble complexes at narrow ranges of potassium chloride concentrations [14] and cannot be separated by anion-exchange chromatography [15, 17]. IR spectra can be used as an additional but not absolute criterion because the shapes and intensities of the absorption bands are strongly influenced by the preparation of the sample [18].

Cystocarpic and tetrasporangial plants of *G. skottsbergii* biosynthesize different and complex systems of carrageenans. Haploid individuals produce similar amounts of κ/ι- and μ/ν-like carrageenans (Tables 1 and 3) while diploid individuals only synthesize λ-carrageenans (Tables 2 and 4), in agreement with the findings for other members of the Gigartinaceae [1–6], with possibly a small amount of the ω-structure (Table 2, 1 T_7) [15, 17]. The simultaneous production of μ/ν- and κ/ι-carrageenans is consistent with the hypothesis that the former are the biological precursors of κ/ι-carrageenans [19, 20].

Tetrasporangial and cystocarpic plants of *Chondrus crispus* have been reported to contain minute contamination with the alternate carrageenan and this was explained as being due to the presence of haploid tetraspores in the diploid tetrasporophytes and of diploid carposporophytic tissue and spores in the haploid gametophytes [21]. The tetrasporangial and cystocarpic plants of *G. skottsbergii* also contain carrageenans (1 T_7, Table 2; see 1 C_1T_3 and 1 C_3T_4, Table 3) which due to some of their characteristics (potassium chloride solubility and/or analytical data) could be considered alternate to their systems; however, their IR spectra show that they have a distribution of sulphate groups similar to that of the other members of their systems suggesting that these products could be considered structural variations of the major members of the system.

In a previous paper, the presence of λ-carrageenans insoluble in potassium chloride solutions has been reported [14]; data shown in Table 2 confirm this fact suggesting that λ-structures can also form junction zones and potassium-specific aggregates.

In spite of the fact that both nuclear phases of Gigartinaceae contain a sulphohydrolase which converts 4-linked galactose 6-sulphate and 2,6-disulphate units to the corresponding 3,6-anhydrogalactose residues, this reaction only takes place with the γ-, μ-, and ν-precursors present in haploid plants [20] and with precursors of the ω-carrageenans in the diploid stage. This enzyme is inhibited by λ-carrageenans suggesting that sulphation on C-2 of the 3-linked unit is deleterious. Inhibition would explain why in pure λ-carrageenans shown in Table 2 appears a small percentage of 3,6-anhydrogalactose (*ca* 1%) considering that λ-carrageenans usually contain minor amounts of non-sulphated 3-linked units.

EXPERIMENTAL

Samples of *G. skottsbergii* were collected in Bahia Camarones (Provincia de Chubut) and sorted in the Instituto Nacional Patagonico (Puerto Madryn, Chubut).

General. Galactose was analysed by the PhOH–H_2SO_4 method [22] without previous hydrolysis of the polysaccharide. Galactose content was corrected for the presence of 3,6-anhydrogalactose, which was determined independently by the resorcinol method [23]. Sulphate was determined by the method of ref. [24]. C-6 sulphate was analysed according to ref. [25]. Optical rotations were measured using 0.2–0.4% solns in 0.1 M NaCl. Solns were equilibrated in the polarimeter tube for 16 hr before determinations; the turbid solns were centrifuged off before measurements were made. IR spectra were recorded using a polysaccharide film.

For GC sugars, obtained from the polysaccharide by hydrolysis with 0.5 M H_2SO_4 during 8 hr at 95°, were transformed into their corresponding alditol acetates. A 30 m × 0.25 mm i.d. fused-silica WCOT column coated with SP-2340 (film thickness 0.20 μm) was used. Chromatography was performed at 230°. The inj temp and FID temp. were 250°. He was used as carrier gas at a flow rate of 1.0 ml/min with a split ratio of 100:1.

Extractions. Cystocarpic and tetrasporangial plants (51.7 and 26 g), previously milled, were extracted with H_2O (2.5 and 1.7 l) with mechanical stirring for 16–24 hr at room temp. The residue was removed by centrifugation and the supernatant poured into 3 vols of *iso*-PrOH (7.5 and 5.0 l), where the polysaccharide pptd as long fibres. The liquors were decanted and the product pressed in filter paper and dried by solvent exchange (EtOH and Et_2O) and finally *in vacuo*. Both residues were extracted twice more. The yields for the first and second extractions were 49.1 and 13.9% for cystocarpic plants, and 43.2 and 7.6% for tetrasporangial plants. For both plants the products from the third extraction were negligible. Crude products from the first extn of the cystocarpic and tetrasporangial plants were analysed by GC of alditol acetates. Galactose and glucose were determined as the only acid-stable sugars: 89.7 and 10.3% for the cystocarpic product and 80.0 and 20.0% for the tetrasporic product. Purification by reprecipitation in *iso*-PrOH and further GC analysis showed only the presence of galactose.

Fractionation of carrageenans with potassium chloride. The pptn curves of carrageenans were determined by the turbidimetric method of ref. [26]. For prep. fractionation, the polysaccharide (1.0–15.0 g) was dissolved in H_2O (0.4–6.0 l, 0.25%). Solid, finely-divided KCl was added in small portions with constant and violent mechanical agitation, so that the concn was increased by 0.01–0.10 M each time. After each addition the stirring was continued for 16 hr to ensure equilibration of the system, the upper limit of KCl concn was 2.0 M. The ppts as well as the residual solns were dialysed, concd and freeze-dried.

Alkaline treatment of fractions. For analytical treatment, the sample (150–300 mg) was dissolved in H_2O (75–150 ml) and $NaBH_4$ (8–16 mg) added. After 24 hr at room temp, 3 M NaOH was added (37.5–75.0 ml) with a further quantity of $NaBH_4$ (4–8 mg). The soln was heated at 80° and the content of 3,6-anhydrogalactose determined on the samples removed at intervals; heating was continued until a constant value was obtained for 3,6-anhydrogalactose content. The soln was then cooled to room temp, dialysed, concd and freeze-dried. For prep. treatment 1.0–1.5 g of sample were used.

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