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The system of agaroids and carrageenans from the soluble fraction of the tetrasporic stage of the red seaweed *Iridaea undulosa*

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The fraction of the carrageenans from the tetrasporic stage of *Iridaea undulosa* soluble in potassium chloride was subfractionated by ion-exchange chromatography on an anion exchanger diluted with gel permeation media. The system under analysis consists of a mixture of λ-carrageenans and variant agaroids. The latter contain large amounts of 3-linked 6-substituted units and galactose side chains. In some molecules, D-glucose units replace D-galactose ones. © 1997 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

L-galactose-containing galactans were found in the 2 M potassium chloride soluble fractions of cystocarpic *Gigartina skottsbergii* after alkaline treatment of the κ/ι- and partially cyclized μ/ν-carrageenans, and further fractionation with potassium chloride (Ciancia *et al.*, 1993). Galactans with similar characteristics have been isolated from cystocarpic samples of other Gigartinaceae (Craigie & Rivero-Carro, 1992). In a previous paper, the structures of the major carrageenans from cystocarpic and tetrasporic stages from *Iridaea undulosa* were thoroughly studied and the presence of a minor fraction containing L-galactose was reported in the tetrasporic stage (Stortz & Cerezo, 1993). We now report the fractionation and methylation analysis of the 2 M potassium chloride soluble products from tetrasporic *Iridaea undulosa*.

MATERIALS AND METHODS

The collection of seaweed, extraction and fractionation of carrageenans to obtain fraction T_3 was reported elsewhere (Stortz & Cerezo, 1993).

Fractionation

The first fractionation attempt was carried out on a DEAE-Sephadex A-50 column (1.0×18 cm) with 15 mg of T_3. Water was used as first eluant and increasing concentrations of NaCl were applied until no phenol-sulfuric-acid-positive material (Dubois *et al.*, 1956) eluted from the column. The upper concentration was 4 M. Finally, the gel was left overnight in 4 M NaCl and boiled for 2 h (Stortz & Cerezo, 1991). After centrifugation, the supernatant was assayed by the phenol-sulfuric-acid method (Dubois *et al.*, 1956).

In a second attempt, DEAE-Sephadex A-50 was equilibrated with 0.2 M NaCl. To 10 ml of the slurry, 500 mg of Sephadex G-100 was added together with 0.2 M NaCl solution. The gel was swollen for 1 h and a column (1.0×16 cm) was filled. After eluting with 0.2 M NaCl, increasing concentrations of sodium chloride were used. In another experiment the same procedure was utilized, but 6.5 ml of DEAE-Sephadex A-50 was mixed with 900 mg of Sephadex G-100.

For the preparative fractionation, a column (1.5×32 cm) was filled with the mixture (32 ml DEAE Sephadex A-50 stabilized in 0.2 M NaCl and boiled with 1.8 g of Sephadex G-100 in sufficient amount of 0.2 M NaCl solution). 221.3 mg of fraction T_3,

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previously dissolved in 0.2 M NaCl, was sampled onto the column. Fractions of 3 ml were isolated and aliquots were assayed by the phenol-sulfuric-acid method (Dubois *et al.*, 1956). After obtaining blank readings, the eluant was replaced by another with higher NaCl concentration.

General and analytical methods

Total carbohydrates were assayed by the phenol-sulfuric-acid method (Dubois *et al.*, 1956) without previous hydrolysis. Sulfate was determined by the method of Dodgson (1961) modified by Dodgson & Price (1962). Optical rotations were measured using 0.4–0.6% solutions in 0.1 M NaCl. Methylation analysis was carried out using the Hakomori method on the triethylammonium salts, as described previously for λ-like carrageenans (Stortz & Cerezo, 1993). The methylated polysaccharides were hydrolyzed (45% formic acid, 100°C, 16 h) and analyzed as their acetylated aldononitriles. GC-MS was used to confirm the identity of some peaks as those corresponding to the methylated glucoses. The configurations of the constituting monosaccharides were determined as their acetylated aminoalditols after reaction with (*S*)-1-amino-2-propanol/sodium cyanoborohydride and further acetylation, as described by Cases *et al.* (1995). In this reaction, each enantiomeric sugar is converted in a single diastereomeric derivative, amenable to GC separation and quantitation.

RESULTS

In a previous paper, the isolation of fraction T_3 was reported (Stortz & Cerezo, 1993). This fraction corresponds to the portion soluble in 2 M potassium chloride of the tetrasporic carrageenans from *Iridaea undulosa* and amounted to only 8.6% of the total product extracted from the seaweed. Its characteristics were not those expected for a carrageenan: the fraction contained around 30% of its galactose as the L-isomer, low optical rotation (11.2°) and an unusual methylation pattern in which important amounts of 2,4-di-, 2,6-di-, 2,3,4,6-tetra- and significant proportions of two tri-O-methylgalactoses coexist with the products expected for the methylation of an λ-carrageenan (Stortz & Cerezo, 1993). Fraction T_3 contains other sugars besides D- and L-galactose (76.1 moles/100 moles): xylose (3.3%), glucose (11.3%), mannose (1.6%), rhamnose (2.2%), fucose (1.4%), 3-O-methylgalactose (2.8%) and 4-O-methylgalactose (1.2%). It contains around 73 moles of sulfate per 100 moles of sugars, from which about a quarter correspond to base-cyclyzable 6-sulfate (Stortz & Cerezo, 1993).

Attemps to fractionate T_3 by ion-exchange chromatography on DEAE-Sephadex A-50 using increasing concentrations of sodium chloride (Cases *et al.*, 1992) led to some separation, with most of the eluted materials at NaCl concentrations between 0.85 and 1.5 M. However, the total recovery was low (35%) and even after boiling the gel with 4 M NaCl (Stortz & Cerezo, 1991) only a further 8% of the original material was solubilized.

To increase the yield of eluted products, the ion exchange resin was diluted with Sephadex G-100. This ion-exchange chromatography enabled the isolation of *ca.* 10 fractions with most of the elution at 1.4–2.0 M NaCl concentrations and an estimated 85% yield. With a further dilution of the anion exchanger, similar results were obtained.

Preparative anion-exchange chromatography gave rise to the pattern shown in Fig. 1. Although this situation is similar to the analytical ones, in this case an important fraction was eluted at low concentrations of sodium chloride (Fig. 1). Eight fractions were isolated, with a total recovery from the column of 55%. Their yields and analysis are reported in Table 1. The optical rotations were plotted against the percentages of L-galactose and D-glucose plus D-galactose of the fractions. Straight lines were obtained (Fig. 2) with only two points showing large deviations (those corresponding to F5 and F17).

Methylation analysis of the fractions is reported in Table 2.

DISCUSSION

It has long been accepted that carrageenans do not contain L-galactose (Craigie, 1990). Furthermore, this sugar was not expected to appear in carrageenophytes of the Gigartinaceae and Phyllophoraceae families, which biosynthesize different structures according to the stage of the life cycle. Cystocarpic plants produce

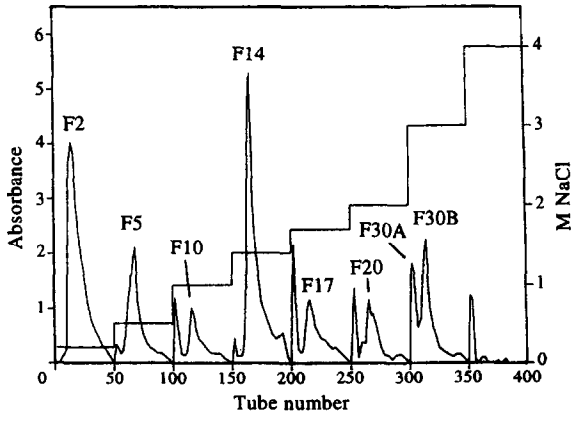

Fig. 1. Elution profile of ion-exchange chromatography on DEAE-Sephadex A-50/Sephadex G-100 of fraction T_3 and acronyms of the isolated fractions.

Table 1. Yields and analysis of the fractions isolated from the soluble tetrasporic carrageenan from *Iridaea undulosa*

	Yield (%)[a]	Sulfate (% SO_3Na)	$[\alpha]_D$ (°)	Residues/100 sugar residues			
				D-Gal	L-Gal	D-Glc	SO_3Na
F2	14.6 (26.3)	21.1	−20.3	49.8	43.0	4.9[b]	85
F5	6.0 (10.8)	6.7	+4.4	13.4	41.1	45.5	36
F10	4.8 (8.6)	15.2	−26.6	36.0	45.1	13.8[b]	58
F14	13.6 (24.6)	23.3	−29.2	53.0	47.0	—	108
F17	3.6 (6.4)	22.3	−10.8	64.8	28.7	6.5	109
F20	2.0 (3.7)	29.1	+37.6	93.1	6.9	—	146
F30A	4.0 (7.2)	19.9	+10.6	76.2	21.5	2.3	124
F30B	6.8 (12.3)	27.8	+43.7	100	tr.	tr.	162

[a]Percentage of the recovered, in parentheses.
[b]F2 and F10 still contain 2.3 and 5.1% of L-rhamnose, respectively.

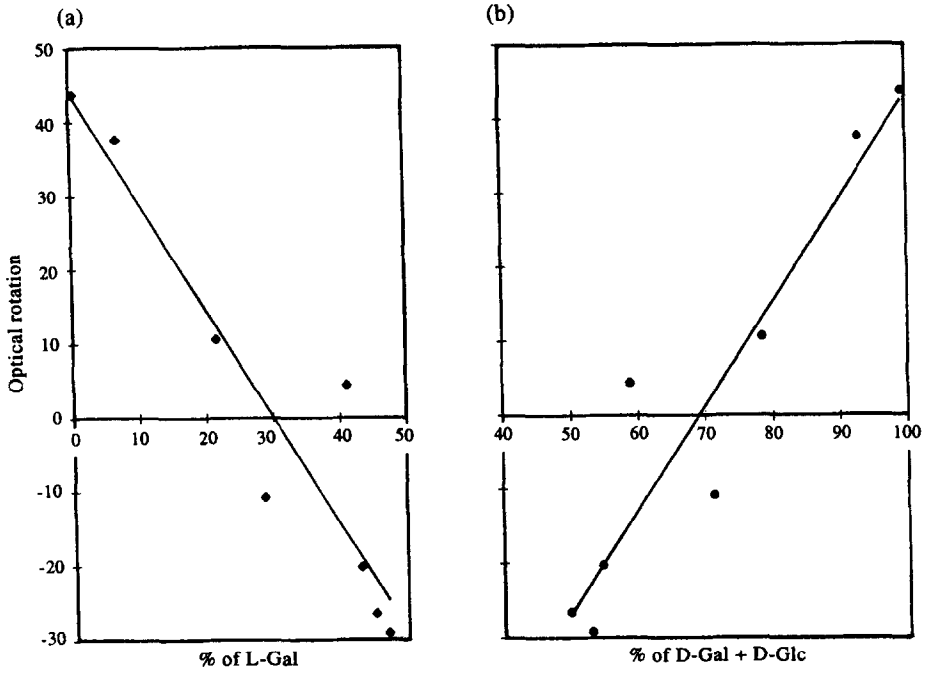

Fig. 2. Plots of the optical rotation vs. the contents of (a) L-galactose and (b) D-galactose + D-glucose.

carrageenans of the κ-family, whereas tetrasporic samples yield λ-carrageenans (Stortz *et al.*, 1997). This simple picture has been complicated by recent reports of the identification of minor amounts of L-galactose-containing galactans in carrageenophytes (Craigie & Rivero-Carro, 1992; Stortz & Cerezo, 1993; Ciancia *et al.*, 1993).

Although traditional anion-exchange chromatography was of little success for the fraction soluble in 2 M potassium chloride of the tetrasporic carrageenans of *Iridaea undulosa*, the sample produced a system of different polysaccharides when submitted to fractionation with the anion exchanger diluted with regular, neutral beads of Sephadex G-100 gel. The fractionation, standard analysis and methylation results (Fig. 1, Tables 1 and 2) show that the system was composed of:

(1) λ-Carrageenans (F30B). The methylation analysis agrees with that expected for this polysaccharide. Substantial amounts of 2,3-di- and 3-mono-O-methylgalactoses agree with the slow cyclization rate reported for λ-carrageenans (Stortz & Cerezo, 1993).

(2) Mixtures (or hybrids) of λ-carrageenans and other polysaccharide/s (F30A and F17). The λ-structures are indicated by the presence of 4,6-di-, 2,3-di- and 3-O-methylgalactoses, though L-galactose-containing disaccharides represent about one-half of the samples. Some other units corresponding to the variant polysaccharide/s are: non-sulfated 3- and 4-linked galactoses, and 3-linked 6-substituted units. This last one is characteristic, for instance, of corallinans (Cases *et al.*, 1994). However, the main one is that indicated by 2,6-di-O-methylgalactose,

Table 2. Methylation analysis of the fractions (residues/100 sugar residues)

Sugar	F2	F5	F10	F14	F17	F30A	F30B	T_3[b]
Galactose								
2,3,4,6-	10.6	21.7	16.6	2.6	7.0	4.2	tr.	12.3
2,4,6-	7.2	1.3	12.2	36.6	16.1	10.2	1.7	8.3
3,4,6-	3.5	7.6	4.6	—	tr.	1.2	—	1.3
2,3,6-	7.4	4.1	7.8	11.6	12.6	8.5	1.7	5.7
2,3,4-	1.4	tr.	2.4	tr.	1.9	tr.	1.6	—
2,6-	15.4	8.1	11.2	6.9	18.3	17.4	3.3	14.2
4,6-	8.6	—	2.2	3.7	17.4	22.2	52.8	9.3
3,6-	tr.[a]	—	—	5.8	1.3	4.8	3.6	—
2,4-	19.1	4.2	19.9	19.1	8.9	6.9	—	22.2
6-	9.9	—	7.4	8.1	2.2	6.4	7.0	7.0
2,3-	3.2	1.2	2.3	2.5	7.1	5.3	10.8	3.8
2-	2.8	tr.	1.2	1.7	4.3	3.2	—	1.5
3-	1.0	—	—	—	tr.	4.8	16.7	tr.
4-	1.0	—	—	—	—	—	—	tr.
Glucose								
2,3,6-	5.4	36.0	8.5	tr.	1.9	3.9	—	9.3
2,6-	2.7	14.5	3.8	—	—	—	—	3.0

[a]Amounts of less than 1% are indicated as traces (tr.).
[b]The composition of T_3 is shown for comparison (Stortz & Cerezo, 1993). It also contained 2,3,4-tri-O-methylxylose (2.1%).

typical of 4-sulfated, 3-linked units in carrageenans of the κ-family, but also assigned to 4-linked, 3-substituted units in corallinans (Cases *et al.*, 1994), a pattern common to other red seaweed galactans (Furneaux & Stevenson, 1990; Usov & Elashvili, 1991). Also, small amounts of side chains (usually not present in carrageenans) appear.

(3) Fractions F2, F10 and F14. These fractions also contain the variant structure, but only small amounts of the λ-carrageenan, if at all. 3-linked, 6-substituted residues, which occur in corallinans, are important units (Cases *et al.*, 1994). Glucose appears methylated only in 2,3,6- and 2,6-.

(4) Fraction F5. This fraction is highly atypical. Although no accurate structure can be assessed for this fraction, the large proportions of D-glucose replacing major amounts of D-galactose and its low sulfation degree (36% for the original fraction, 6% according to methylation analysis) indicate large differences from the other fractions.

Methylation yields polysaccharide derivatives for which branching units appear underestimated compared to what would be expected from sulfate analysis. This can be explained, at least in part, by partial cyclization for λ-like products. However, other unusual desulfations may have also occurred.

The direct relationship between optical rotation and the percentages of L-galactose and of D-galactose + D-glucose (Fig. 2) suggests that their structural influence is similar in all the fractions. Only F5 and F17 deviate slightly from this behavior. In the first case, this could be the result of its large proportion of glucose, which may possibly have partial α-configuration.

In summary, the 2 M potassium chloride soluble fraction obtained from tetrasporic samples of *Iridaea undulosa* is composed of λ-carrageenans and other polysaccharide/s with structures that differ substantially from those of known carrageenans. The main deviating factors are the presence of L-galactose and D-glucose as constituting monosaccharides, residues of 3-linked, 6-substituted and possibly 4-linked, 3-substituted units, and galactose side chains. In some fractions, 4-linked D-glucose units replace D-galactose units. In the polysaccharides from *Anatheca dentata* (Solieraceae, Gigartinales), Nunn *et al.* (1981) have already found α-D-glucuronic acid residues replacing the 3-linked β-D-galactose ones, while those of *Lomentaria catenata* (Rhodymeniales) carry side chains of glucose and glucuronic acid (Takano *et al.*, 1994). Notwithstanding the presence of glucose and the virtual absence of monomethylated galactoses, the deviant polysaccharide has a structure very similar to that encountered for the polysaccharides of *Corallina officinalis* (Cases *et al.*, 1994; Stortz *et al.*, 1997).

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