Carbohydrate Research, 172 (1988) 139–146
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

THE λ-COMPONENTS OF THE "INTERMEDIATE" FRACTIONS OF THE CARRAGEENAN FROM *Iridaea undulosa*

CARLOS A. STORTZ AND ALBERTO S. CEREZO*

Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales (UBA), Pabellón 2, Ciudad Universitaria, 1428 Buenos Aires (Argentina)

Received May 28th, 1986; accepted for publication in revised form, June 19th, 1987)

ABSTRACT

The "intermediate" fractions of the carrageenan of *Iridaea undulosa*, which are precipitated at 1.20–1.25M, 1.35–1.40M, and 1.55–1.65M concentrations of potassium chloride, are mixtures of μ/ν-carrageenans and λ-carrageenans. The λ-carrageenans contain mainly 3-linked, 2-sulfated (33.5–39.0%) and 2,6-disulfated (5.6–11.8%) galactose units, together with 4-linked, 2-sulfated (1.8–5.9%), 6-sulfated and/or 2,6-disulfated (39.0–40.1%), and other 2,6-disubstituted (3.7–4.3%) galactose residues. The presence of this last unit suggests that some C-6 atoms must be either branching points or blocked by glycosidic linkages. When the compositions of these gel-forming λ-carrageenans are compared with those of the "soluble" λ-carrageenans from the same seaweed, it is clear that in the latter ones there is a higher diversification of the structural units and more "solubility-promoting" residues.

INTRODUCTION

The terms ι, κ, λ, μ, ν, *etc.* have been used for defined structures of carrageenans[1], and the names "insoluble", "intermediate", and "soluble" respectively[2] serve as an operational classification of the fractions of carrageenans that are precipitated at concentrations of potassium chloride below 0.125M, and in the range of 0.125–2M, and that are soluble at all concentrations of this salt.

The carrageenan of *Iridaea undulosa* is composed of major proportions of intermediate fractions and lesser proportions of a soluble fraction[3]. These intermediate carrageenans could be separated into two groups, namely, (*a*) those products soluble at 0.125M but that are precipitated in the range of 0.125–1.05M KCl, which have characteristics of κ/ι-carrageenans[3,4], and (*b*) the fractions soluble at 1.05M concentration, but which are precipitated at higher potassium chloride concentrations and have a λ-type of composition[3,4].

In earlier work, we studied the soluble fraction of the carrageenan of *Iridaea undulosa* and found that it contains both μ/ν-like and λ-like carrageenans, with

* To whom correspondence should be addressed.

0008-6215/88/$03.50

regions of structural irregularity formed by masked and "unusual" units[5].

We now report structural studies on the alkali-treated λ-components of the intermediate carrageenans that are precipitated at 1.20–1.25M, 1.35–1.40M, and 1.55–1.65M potassium chloride concentrations. These results, taking into account the known alkaline conversion of the 4-linked, 6-sulfated and 2,6-disulfated galactose residues into 3,6-anhydrogalactose units, allow some inferences as to the structures of the original λ-carrageenans[6] to be drawn.

EXPERIMENTAL

General and analytical methods were performed as already described[5]. Isolation of the carrageenan of *Iridaea undulosa*, and separation of the fractions precipitated at 1.20–1.25M (fraction D), 1.35–1.40M (fraction C), and 1.55–1.65M (fraction B) potassium chloride, were carried out as already reported[3].

Alkali treatment. The carrageenan fractions D, C, and B (250 mg each) were each dissolved in water (100 mL) and reduced overnight with sodium borohydride (100 mg). Sodium hydroxide (3M, 50 mL) and sodium borohydride (100 mg) were added and the solutions were heated for 2–3 at 80°. Previous controls of these treatments in which the 3,6-anhydrogalactose content was monitored as a function of time had shown that maximum values are obtained after 2–3 h of heating, and that they remain constant for at least 3 h more. The solutions were then dialyzed, and freeze-dried, producing the alkali-treated carrageenans (DT, CT, and BT, respectively) in 86.6–95.2% yield (see Table I). The alkali-treated carrageenans were fractionated by precipitation with 0.4M potassium chloride, giving DT_1, CT_1, and BT_1 respectively (see Table I) and on increase to 2M potassium chloride, DT_3, CT_3, and BT_3 remained soluble and were isolated by dialysis and freeze-drying (see Table I).

Methylation analysis. — Methylation of the alkali-treated, 2M KCl-soluble carrageenans from fractions D, C, and B (DT_3, CT_3 and BT_3, respectively) was carried out by the four-step Haworth procedure at room temperature as previously described[5]. Partially methylated sugars resulting from hydrolysis of the permethylated carrageenans with 5% formic acid for 16 h at 100° were analyzed and identified by g.l.c. and computerized g.l.c.–m.s. through combined use of the acetylated alditol and acetylated aldononitrile derivatives. The use of the first procedure resolves all the partially methylated galactoses except 2,3,6-tri-*O*-methyl- and 3,4,6-tri-*O*-methyl-galactose; 2,6-di-*O*-methyl- and 4,6-di-*O*-methyl-galactose; and 3-*O*-methyl-galactose and the free sugar[5]. When the acetylated aldononitrile derivatives were used, all the methylated galactoses were resolved, except 4,6-di-*O*-methyl- and 3,6-di-*O*-methyl-galactose; and 2,3-di-*O*-methyl-, 2,4-di-*O*-methyl-, and 6-*O*-methyl-galactose[7]. G.l.c. analyses were performed in Hewlett–Packard Research Gas Chromatographs, models 5530 and 5840 A, equipped with flame-ionization detectors and glass columns (180 × 0.2 cm) containing 3% of ECNSS-M on Gas Chrom Q (100–120 mesh), operated isothermally at 180°, with an injection-chamber

TABLE I

YIELDS AND ANALYSES OF THE ALKALI-TREATED CARRAGEENANS AND THEIR SUBFRACTIONS

Fraction	*Identifying characteristic*	*Yield (%)*	*Gal: 3,6-AnGal: sulfate (molar ratio)*	*Residues/100 sugar units*	
				Sulfate	*3,6-AnGal*
D	insol.in 1.20–1.25M KCl	15.0[a]	1:0.07:1.36	127	7
DT	alkali-treated D	86.6[b]	1:0.64:1.42	87	39
DT_1	fract.of DT insol.in 0.4M KCl	14.9(17.7)[c]	1:1.07:1.46	71	52
DT_3	fract.of DT sol.in 2M KCl	69.2(82.3)[c]	1:0.67:1.48	89	40
C	insol.in 1.35–1.40M KCl	7.2[a]	1:0.07:1.37	128	7
CT	alkali-treated C	95.2[b]	1:0.71:1.51	88	42
CT_1	fract.of CT insol.in 0.4M KCl	20.3(24.3)[c]	1:1.08:1.20	58	52
CT_3	fract.of CT sol.in 2M KCl	63.1(75.7)[c]	1:0.64:1.54	94	39
B	insol.in 1.55–1.65M KCl	13.2[a]	1:0.06:1.34	126	6
BT	alkali-treated B	89.3[b]	1:0.65:1.65	100	39
BT_1	fract.of BT insol.in 0.4M KCl	16.8(24.5)[c]	1:0.74:1.31	75	43
BT_3	fract.of BT sol.in 2M KCl	51.8(75.5)[c]	1:0.65:1.60	97	39

[a] Yield from fractionation of parent carrageenan. [b] Yield from alkaline treatment. [c] Yield from fractionation of the alkali-treated carrageenans (in parentheses, percent of the total recovered).

temperature of 210°. Computerized g.l.c.–m.s. was performed in a glass column (120 × 0.2 cm) of 3% of ECNSS-M on Gas-Chrom Q (100–120 mesh) at 180°, with helium as the carrier gas (30 mL/min) in a Varian Series 1440 chromatograph connected to a Varian MAT CH7A mass spectometer.

RESULTS

The three intermediate fractions studied were precipitated at very sharp ranges of potassium chloride concentration, namely, 1.20–1.25M (fraction D), 1.35–1.40M (fraction C), and 1.55–1.65M (fraction B), at room temperature. It is noteworthy that precipitations at lower KCl concentrations were achieved at lower temperatures, and that the carrageenans were not precipitated at any concentration of sodium chloride.

Analyses of the fractions are given in Table I; the molar ratios G:A:S (Gal:3,6-AnGal:sulfate) are those of typical λ-carrageenans. The alkaline treatment of B, C, and D produced, in yields of 86–95% (see Table I), the corresponding derivatives BT, CT, and DT. An increase of 3,6-anhydrogalactose units was concomitant with an almost stoichiometric decrease of sulfate groups for the three samples (see Table I). The fractionation of the alkali-treated carrageenans with potassium chloride yielded, in the three cases, a fraction insoluble in 0.4M KCl (BT_1, CT_1, and DT_1, 18–25% yields; see Table I) together with others soluble in 2M KCl (BT_3, CT_3, and DT_3, 75–82% yields; see Table I). No material was precipitated at KCl concentrations between 0.4 and 2M. The analyses of the six alkali-treated carrageenans are given in Table I; the respective compositions of BT_1, CT_1, and DT_1 suggest a structure of the hybrid κ/ι-type, in agreement with their precipitation behavior. The molar ratios G:A:S of BT_3, CT_3, and DT_3 (see Table I) suggest that most of the 3,6-anhydro units were 2-sulfated, indicating a preponderance of the 4-linked, 2,6-disulfated galactose residues over the 6-sulfated in B, C, and D, in agreement with their analyses (see Table I) and with the "ideal" composition of a λ-carrageenan.

The compositions of the permethylated BT_3, CT_3, and DT_3 carrageenans as partially methylated galactoses are given in Table II. On the basis, when possible, of the alternating (1→3)- and (1→4)-linked model, most of the methylated galactose derivatives could be assigned to structural units (see Table III). The major methylated galactose derivative was, in all three cases, 4,6-di-*O*-methylgalactose (56.2–64.0%), indicating the presence of major proportions of the 3-linked 2-sulfated galactose unit. The presence of 3,6-di-*O*-methylgalactose (3.1–9.8%) and 4-*O*-methylgalactose (9.4–19.8%) suggested 4-linked, 2-sulfated, and 3-linked, 2,6-disulfated galactose units. The presence of 3-*O*-methylgalactose (6.0–7.2%) in an alkali-treated carrageenan (see Discussion) is noteworthy. All of the other methylated galactose derivatives could be assigned to 3-linked or 4-linked residues, and both linkages, after including the 3,6-anhydro units in the calculated composition (see Table III), were found in similar proportions, in agreement with the alternating, (1→3)- and (1→4)-linked model.

TABLE II

ACID-STABLE PARTIALLY METHYLATED GALACTOSES FROM PERMETHYLATED CARRAGEENANS DT_3, CT_3, AND BT_3

Carrageenan	*Mole percent of the galactose having methyl groups at the position indicated*[a]												
	2,3,4,6	*2,4,6*	*2,3,6*	*3,4,6*	*2,6*	*4,6*	*3,6*	*2,4*	*6*	*2*	*3*	*4*	*none*
DT_3	1.8	1.9	tr.	tr.	tr.	62.9	9.8	1.2	2.4	tr.	6.5	9.4	1.0
CT_3	2.2	tr.	tr.	tr.	tr.	64.0	7.3	1.3	3.7	tr.	6.0	11.7	1.5
BT_3	tr.	3.5	2.6	tr.	1.3	56.2	3.1	1.4	2.4	tr.	7.2	19.8	1.5

[a] Percentages lower than 1% are given as "trace" (tr.)

TABLE III

THE COMPOSITION OF THE KCl-SOLUBLE, ALKALI-TREATED CARRAGEENANS[a]

Carrageenan	*Percentage of the sugar having sulfate at the positions indicated*														
	3-linked galactose							*4-linked galactose*				*3,6-AnGal*[c]	*Other units*		
	none	*2*	*4*	*6*	*2,6*	*2,4*	*4,6*	*none*	*2*	*6*[b]	*2,6*[b]		*Gal*		*Xyl*
													2L[d]	*T*[e]	*T*[e]
DT_3	1.1	37.7	tr.	tr.	5.6	1.4	tr.	tr.	5.9	—	3.9	40.1	tr.	1.1	—
CT_3	tr.	39.0	tr.	tr.	7.1	2.3	tr.	tr.	4.5	—	3.7	39.0	tr.	1.3	—
BT_3	2.1	33.5	tr.	tr.	11.8	1.4	tr.	1.5	1.8	—	4.3	39.4	tr.	tr.	—
AsT_3[a]	4.3	28.4	6.1	2.3	9.0	9.8[f]	tr.	1.8	7.4	9.8[f]	3.0	20.6	1.5	3.1	1.0
AiT_3[a]	3.8	22.8	4.0	1.1	7.8	4.2	1.3	2.7	8.5	2.4	1.8	32.4	2.4	2.3	1.7

[a] For comparison, the composition of the alkali-treated λ-components of the "soluble" carrageenan (AsT_3 and AiT_3; see ref. 5) are included. [b] The substituent on C-6 cannot be a sulfate group. [c] 3,6-Anhydrogalactose plus its 2-sulfated derivative, originated from 4-linked, 6-sulfate and 2,6-disulfated galactose units in the original λ-carrageenans. [d] 2-Linked, non-sulfated. [e] Non-sulfated terminal units. [f] The figure shows the contribution of both sugars, mainly the 3-linked unit.

DISCUSSION

Previous studies[5] on the potassium chloride-soluble carrageenan from the red seaweed *Iridaea undulosa* showed that its composition could be expressed in terms of μ/ν- and λ-structures if the molecules were described by using the "interrupted chain sequence" model[8].

The study of λ-like structures is possible due to separation from the μ/ν-like ones (which usually accompany them[1,6]) through the alkaline treatment which transforms, by the known cyclization mechanism, the μ/ν-structures into κ/ι ones, which can be precipitated at low concentrations of potassium chloride[8]. By the same reaction, the alkali transforms the λ-molecules into carrageenans that are completely soluble in KCl solutions and which, after separation of the gel-forming material, can be isolated. The study of these alkali-transformed λ-carrageenans allows, by taking into account the conversion of the 4-linked, 2-sulfated, and 2,6-disulfated galactose units into 3,6-anhydrogalactoses, making inferences as to the structures of the original λ-carrageenans[6].

These intermediate fractions of the carrageenan of *Iridaea undulosa* (fractions B, C, and D) have the composition and i.r. spectra of λ-carrageenans[3], but they contain 15–20% of a μ/ν-component (see Table I). Like the soluble carrageenans from the same seaweed[5], these intermediate fractions are mixtures of two carrageenans, one containing λ-blocks and the other containing μ/ν-blocks. A major difference as regards the components of the soluble fraction is that the λ- and μ/ν-carrageenans from B, C, and D afford gels in potassium chloride solutions. To the best of our knowledge, this is the first report of well characterized λ-carrageenans that are precipitated in KCl solutions, as these compounds were considered to be soluble under such condition[1,9]. The gel formation is specific for potassium, as sodium chloride does not produce precipitation at any concentration or temperature.

The calculated conformation of a λ-molecule resembles a rather flat, extended ribbon having some bending from side to side[1]. The possibility of obtaining oriented fibers of λ-carrageenan[10], the X-ray analysis of which agreed with that conformation, showed that λ-chains can be packed into junction zones. The packing could be stabilized by the potassium counter-ion, which would promote the associateion of the chains in small clusters and, further, into aggregates[11–14].

In order to build up a network structure, a polysaccharide chain must have a kink in the regular sequence[8]. Methylation analyses of the alkali-treated λ-carrageenans suggest the possible presence of (1→6) linkages (see Table III and later); the freedom of rotation between each residue and the next would introduce "flexible joints" in the molecular chain[15], and provide the possibility of nucleation with different chain partners.

The B, C, and D fractions were precipitated in very sharp concentration-ranges of KCl (0.05–0.1M); nevertheless, they were mixtures of λ- and μ/ν-carrageenans; this suggests that both types of molecule, which differ only in the position of sulfation on the 3-linked unit, could form some type of mixed aggregate.

The compositions of BT_3, CT_3, and DT_3 (see Table III) are very similar (with increasing proportions of 3-linked 2,6-desulfated units and decreasing ones of 4-linked 2-sulfated galactose residues) to that of the alkali-treated derivatives of the more-soluble λ-carrageenans (see Table III). In the three cases, there are similar proportions of 2,4,6-trisubstituted galactose unit (3.7–4.3%; see Table III) which, in an alkali-treated product, cannot be a 4-linked 2,6-disulfated galactose. Therefore, C-6-must either be a branching point or be blocked by a glycosidic linkage. The compositions of the permethylated BT_3, CT_3, and DT_3 (see Table II) indicate the presence of only small proportions of end-chain groups, and this indicates the possibility of (1→6) linkages, which were suggested for the soluble carrageenans from the same seaweed[5]. The conclusions based on this and other monomethylated sugars are only tentative, due to the possibility that they resulted from incomplete methylation; nevertheless, the proportions are actually significant, and appear in all of the compounds studied.

When the compositions of these carrageenans are compared with those of the alkali-treated λ-components (AsT_3 and AiT_3) of the soluble carrageenan[5] (see Table III), it is clear that, in the latter two, there is a higher diversification of the structural units and also that there are present "solubility-promoting" residues that do not appear in the gel-forming λ-carrageenans.

The "interrupted chain sequence" model of carrageenans describes a molecule in which sequences having repeating regularity are separated by regions having no regularity at all. The gel-forming λ-carrageenans from the intermediate fractions of *Iridaea undulosa* can be described, in terms of this model, as a linear polymer with galactose linked alternately α-(1→3) and β-(1→4), in which the disposition of the sulfate groups produces segments having the regular structure defined for the "ideal" λ-carrageenan (75–80% of the molecule) and zones with different, "irregular" sulfation patterns. These irregular patterns are produced by adding, in the 3-linked 2-sulfated galactose units, a new sulfate group at C-4 or -6 (mainly the latter, already reported by Parolis for the polysaccharide of *Pachymenia hymantophora*[16]) and in the 4-linked 2,6-disulfated galactose residues by removing the 6-sulfate. Irregular zones may also be produced by changing a small proportion of (1→4)-linkages (~4%) into (1→6) linkages.

ACKNOWLEDGMENTS

We are indebted to Dr. María C. Matulewicz for the gift of samples B, C, and D, and to the UMYMFOR (FCEyN-CONICET) for technical assistance. This work was supported by grants from CONICET and UNESCO (Rostlac/211.914.5).

REFERENCES

1 D. A. Rees, *Adv. Carbohydr. Chem. Biochem.*, 24 (1969) 267–332.
2 A. J. Pernas, O. Smidsrød, B. Larsen, and A. Haug, *Acta Chem. Scand.*, 21 (1967) 98–110.
3 M. C. Matulewicz and A. S. Cerezo, *J. Sci. Food Agric.*, 31 (1980) 203–213.

4 M. C. MATULEWICZ AND A. S. CEREZO, *Phytochemistry*, 19 (1980) 2639–2641.
5 C. A. STORTZ AND A. S. CEREZO, *Carbohydr. Res.*, 145 (1986) 219–235.
6 N. S. ANDERSON, T. C. S. DOLAN, C. J. LAWSON, A. PENMAN, AND D. A. REES, *Carbohydr. Res.*, 7 (1968) 468–473.
7 C. A. STORTZ, M. C. MATULEWICZ, AND A. S. CEREZO, *Carbohydr. Res.*, 111 (1982) 31–39.
8 D. A. REES, *Polysaccharide shapes*, Chapman and Hall, London, 1977, p. 65.
9 C. J. LAWSON, D. A. REES, D. J. STANCIOFF, AND N. F. STANLEY, *J. Chem. Soc., Perkin Trans. 1*, (1973) 2177–2182.
10 S. T. BAYLEY, *Biochim. Biophys. Acta*, 17 (1955) 194–205.
11 G. ROBINSON, E. R. MORRIS, AND D. A. REES, *J. Chem. Soc., Chem. Commun.*, (1980) 152–153.
12 E. R. MORRIS, D. A. REES, AND G. ROBINSON, *J. Mol. Biol.*, 138 (1980) 349–362.
13 O. SMIDSRØD, I.L. ANDRESEN, H. GRASDALEN, B. LARSEN, AND T. J. PAINTER, *Carbohydr. Res.*, 80 (1980) c11–c16.
14 H. GRASDALEN AND O. SMIDSRØD, *Macromolecules*, 14 (1981) 229–231.
15 D. A. REES AND W. E. SCOTT, *J. Chem. Soc., B*, (1971) 469–479.
16 H. PAROLIS, *Carbohydr. Res.*, 93 (1981) 261–267.