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Gelling galactans from the algal community of *Furcellaria lumbricalis* and *Coccotylus truncatus* (the Baltic Sea, Estonia): a structure-property study

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Abstract

Extraction of *Furcellaria lumbricalis* by potassium hydroxide solution shows that the maximum gel strength of polysaccharides separated is attained by extraction at concentration 0.16 M KOH. Crude extract from this algal species has a complicated monosaccharide composition; a drastic increase of gel strength by alkaline extraction of the polysaccharide mixture cannot be explained through the composition of a major (kappa) fraction described earlier. Alkaline treatment is also suitable for accelerating the extraction of polysaccharides from *Coccotylus truncatus* overlaid with thick epithelium. Viscous galactans from *C. truncatus* are more stable to alkali, their composition is simpler and close to iota carrageenan. The formation of mixed kappa and iota carrageenan gels as a result of simultaneous extraction of both seaweeds causes levelling of rheological properties of different extraction fractions.

Keywords: Kappa carrageenan; Furcellaran; Iota carrageenan; Gelation; Alkaline modification

1. Introduction

Carrageenans, the specific polysaccharides of algae, are contained in various *Rhodophyceae* species, form a family of linear sulphated galactans [1]. Carrageenans from particular seaweed species and/or geographic districts differ considerably in their structure (although have the same building pattern) and rheological properties of solutions or gels and are widely used in the food industry.

All types of carrageenans (denoted by Greek prefixes in the names) are built up of alternating 3-linked β-D-galactopyranose and 4-linked α-D-

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galactopyranose residues, part of the latter may exist as a 3,6-anhydro derivative. Various hydroxyl groups in these polymeric chains may be substituted (sulphated, methylated, etc.) by monosaccharide residues [2]. Red algae contain often non-stoichiometrically sulphated carrageenans. Part of them are known historically as furcellarans, polysaccharide mixtures from *Furcellaria* species [3,4]. Since traditional names like 'carrageenan' or 'furcellaran' do not express the real structure of the galactans, these names are presented side by side with the terms of a new nomenclature [5] in this work.

In our paper, two principal fractions of carrageenan (Fig. 1) called kappa carrageenan (carrageenose 4′-sulphate) and iota carrageenan (carrageenose 2, 4′-disulphate) are studied.

There are numerous publications on the characterization of 'individual' carrageenans [6] and fractions of kappa and iota carrageenans are also studied in comparison and in mixture with themselves [6–10]. But actually, the separation of carrageenan components is very complicated and commercial as well as laboratory samples are far from purity [7].

In addition to the heterogeneity of organic substance, the carrageenans contain a considerable amount of inorganic cations (usually about 10% or more from the mass). The gelling properties of carrageenans depend on the composition of polysaccharic part as well as on the presence of counterions (and co-ions, see review [11]). In this paper, the limits of saturation with potassium ions of the 'furcellaran' (non-stoichiometric carrageenose 4′-sulphate) are characterized. To avoid the traditional treating with potassium chloride, the algae were extracted by potassium hydroxide solution in our study.

Although the carrageenan fractions are widely investigated, the actual composition (and therefore, rheological properties) of algal polysaccharides has often remained unknown. Simple structural models appear frequently to be insufficient for adequate description of real algal galactans. An example is given below.

Gelling galactans of *Furcellaria lumbricalis* f. *aegagrophila* from the algal stratum in Kassari Bay (the Baltic Sea, Estonia) are described in two papers [12,13] (where the former term *Furcellaria fastigiata* has been used). The major galactan component (kappa-fraction) from these algae is isolated and the molar ratio of component sugars is calculated from the ^{13}C NMR spectra [12]. Later, the results are certified by methylation analysis: the galactan consists of residues of D-galactopyranose, its 4-sulphate, 3,6-anhydro-D-galactopyranose and its 2-sulphate according to molar ratio 2:1.5:2.5:0.2, respectively; the share of the fraction labile to alkali is very low [13]. From these results it may be concluded that alkaline treatment has no noticeable effect on the gel strength of this polysaccharide. However, the alkaline modification of natural (non-fractionated) galactans of *Furcellaria lumbricalis* from Kassari Bay is actually possible and it causes a drastic increase in gel strength; its limits are reported in this paper.

Natural sources of carrageenans have been strongly damaged in northern waters during the last decades. So, the resources of *Furcellaria lumbricalis* are essentially lessened in Kattegat (Denmark) [14] and completely destroyed in Puck Bay (Poland) [15]. For that reason, the stratum of these seaweeds in Estonia is the greatest in Europe ([16]) and probably in the world. Summary wet biomass of the algal stratum in Kassari Bay is ≈ 115 thousand tons according to latest investigations (summer 1995) [17]. Another algal species *Coccotylus truncatus* (= *Phyllophora truncata* = *Phyllophora brodiaei*) occurring in the Kassari stratum has not been described earlier. Broadly speaking, galactans of this species belong to iota carrageenan pattern [18,19] and have an industrial use [20].

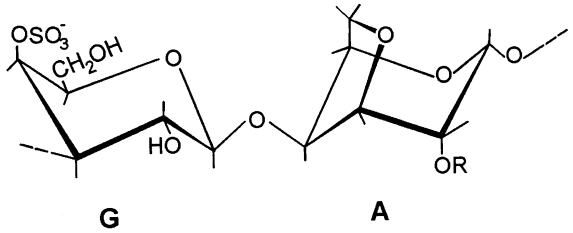

Fig. 1. Disaccharide repeating units of kappa (R represents H) and iota (R is SO_3^-) carrageenan.

The purpose of the present work is to determine the composition and structural features of the gelling galactans from whole water extract of *Furcellaria lumbricalis* and *Coccotylus truncatus*, collected in Kassari Bay. In particular, attention is paid to the changes in the chemical composition and structure, having a considerable effect on the gel strength.

2. Material and methods

2.1. Algae samples

Seaweeds *Furcellaria lumbricalis* and *Coccotylus truncatus* were collected in Kassari Bay (the Baltic Sea, Estonia) by using an aqualung from the depth of 6–8 m in July 1995. The plants were thoroughly washed in tap water and dried in air. When necessary, algae were sorted; no preliminary treatment was made.

2.2. Extraction, precipitation and gel testing

Suspension of algae in 30–50-fold mass of water or potassium hydroxide solution was refluxed above an air bath; time of extraction was counted in the boiling state. For determinations of extraction yields, the concentration of alkali was 0.1 M, for other investigations in the range 0.01–0.32 M. The extract was filtered under rarefying through a porous glass filter (P2, Labklaas, Tallinn, Estonia). Algal polysaccharides were completely precipitated from the extract by adding it to ethanol (95% v/v, 4-fold volume per extract) and washed after filtration with ethanol (80% v/v).

The gel tester was constructed in our laboratory and it has a hemispheric tip of the plunger. The gel strength measurements were done for 1.5% gels in triplicate after gelling in thermostat (20°C, 2 h) and the values of breaking force expressed in g/cm^2.

2.3. Commercial samples

Carrageenan preparations were purchased from Sigma. Kappa carrageenan was a mixed salt form containing 6.8% K^+, 0.6% Na^+ and 2.4% Ca^{2+}. Iota carrageenan (also in salt form) contained 5.4% K, 1.3% Na and 4.2% Ca. The origin and purity of these samples was an object of discussion [7].

Table 1
Monosaccharide composition of summary polysaccharide from *Furcellaria lumbricalis* (% from air-dry matter)

Monosaccharide	Varying range
Xylose	0.63–0.67
2-*O*-Methyl-3,6-anhydrogalactose	0.90–1.0
6-*O*-Methylgalactose	2.0–2.7
3,6-Anhydrogalactose	19–23
4-*O*-Methylgalactose (?)	0.24–0.30
Mannose	0.14–0.24
Glucose	1.6–4.0
Galactose	31–35
Sum of sugars	59–65

2.4. Chemical analysis

The content of 3,6-anhydro-D-galactose was determined colorimetrically by using a resorcinol assay [21], with fructose as the standard sugar. For more complicated determinations (Table 1), a hydrolysis method [22] in the presence of 4-methylmorpholineborane was used by applying of gas–liquid chromatography (a Hewlett-Packard 5890 A chromatograph) for determination of monosaccharides.

The potassium content bound to furcellaran matrix was quantified by sodium tetraphenyl borate [23].

The sulphur content of the samples was determined by classic weight analysis as $BaSO_4$.

2.5. Scanning electron microscopy

Samples of air-dry algae were immersed in liquid nitrogen, cryofractured and dried in air. Then, samples were mounted on specimen stubs with double-sided adhesive tape and coated with gold. The specimens were viewed under a scanning electron microscope (Jeol ISM 840 A) operated at an accelerating voltage of 10 KV.

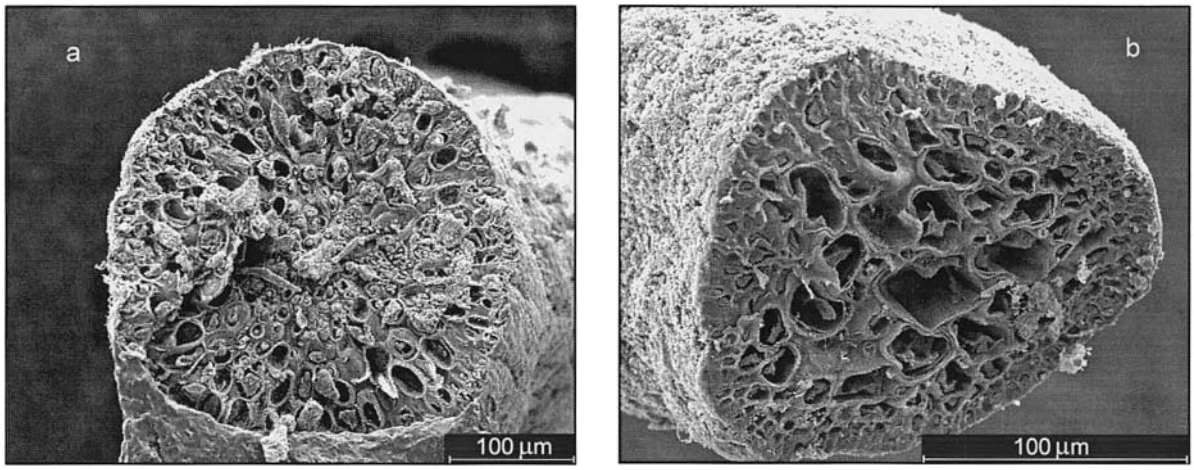

Fig. 2. Scanning electron micrographs of (a) *Furcellaria lumbricalis* and (b) *Coccotylus truncatus* (cross-sections).

2.6. NMR spectroscopy

Proton-decoupled ^{13}C NMR spectra were measured on a Bruker AMX-500 spectrometer for 1–1.5% carrageenan solutions in H_2O at 65°C (furcellarans) or at 80°C (iota carrageenans). A small amount of D_2O was added for the lock signal and about 20 thousand scans were accumulated before the Fourier transform. Chemical shifts were converted to tetramethyl silane scale on the basis of C-6 signal from the galactose subunit having the constant value (61.3 ppm) in these carrageenans [19].

3. Results and discussion

3.1. Yields and dynamics of extraction

3.1.1. Furcellaria lumbricalis

Water extracts 37% and KOH solution 26% of the summary polysaccharide (SP) from the algal mass during 6 h. Further pulverization of the seaweeds does not allow of a correct yield determination. Generally, the process is fast. Comparative fractional extraction in different media suggests that alkali does not accelerate essentially the separating course of galactans.

3.1.2. Coccotylus truncatus

Water extraction gives a yield of 11–13.5% SP from the algal mass during 7 h; extraction is slow and may be continued up to 13 h. The extraction process may be considerably promoted by alkali in medium: already during 3 h the maximum yield 17.5% is achieved. If algae are preliminarily treated during a week in 0.1 M KOH solution, washed to neutral and after that extracted in water, the extraction process will be even more radical, but there is a danger of excessive pulverization of seaweeds.

Different extraction dynamics in various media of two algal species, frequently intertwined with each other in the Kassari stratum, is explicable by their morphological peculiarities (Fig. 2). Presumably, a thick epithelium of *Coccotylus truncatus* inhibits the water extraction of these seaweeds.

3.2. Composition of galactan mixtures

SPs obtained by ethanol precipitation from the algal extract have a heterogeneous composition. In addition to various saccharic components they have a very diverse inorganic part in their composition (unpublished results).

3.2.1. Furcellaria lumbricalis

Monosaccharide composition of SP from these

Table 2
Summary polysaccharides of *Furcellaria lumbricalis*: characteristics after 3-h extraction

Characteristic	Medium	
	Water	0.1M KOH
Molar ratio 3,6-anhydrogalactose/galactose[a]	0.69	0.82
Sulphur content, %	3.3	2.7
Yield, %	33	19
Ash content[b], %	17–20	

[a] Determined according to [22].
[b] At 650°C, 3 h.

algae proved to be much more complicated than described earlier for the major (kappa) component [12,13]. Value ranges for various fractions of extraction (by water and alkali, during 1–6 h) are presented in Table 1.

So, the general monosaccharide composition of SP depends only slightly on extraction conditions. However, a comparison of some characteristic parameters for water and alkaline extraction reveal substantial differences (Table 2).

Viscous polysaccharides from *Coccotylus truncatus* have a low 3,6-anhydrogalactose content (13–15%). The ash content is high, 30–32%. The polysaccharides from this species form a gel neither in the case of water nor alkaline extraction. The sulphur content varies to a slight degree by the extraction medium and fractions, having a mean value 6.3%.

3.3. Structural features

On the basis of ^{13}C NMR spectra, the mixed polysaccharides from *Coccotylus truncatus* have a very permanent structural pattern: various fractions of water extraction have nearly identical spectra during 11 h. Alkaline extraction only slightly clarifies the spectrum of galactans from this algal species.

Fig. 3 is a typical spectrum of iota carrageenan and is close to that of Sigma preparation and similar to the spectrum of polysaccharides from *Phyllophora brodiaei* (from the Black Sea) [19], though they have undergone different isolating procedures.

The ^{13}C NMR spectrum of water-extracted polysaccharides from *Furcellaria lumbricalis* (precipitated by ethanol from the crude extract) is complicated (Fig. 4(a)). However, the product of alkaline extraction and the kappa fraction (insoluble in 0.33 M KCl solution [24]) isolated from the water extract of those algae have similar spectra

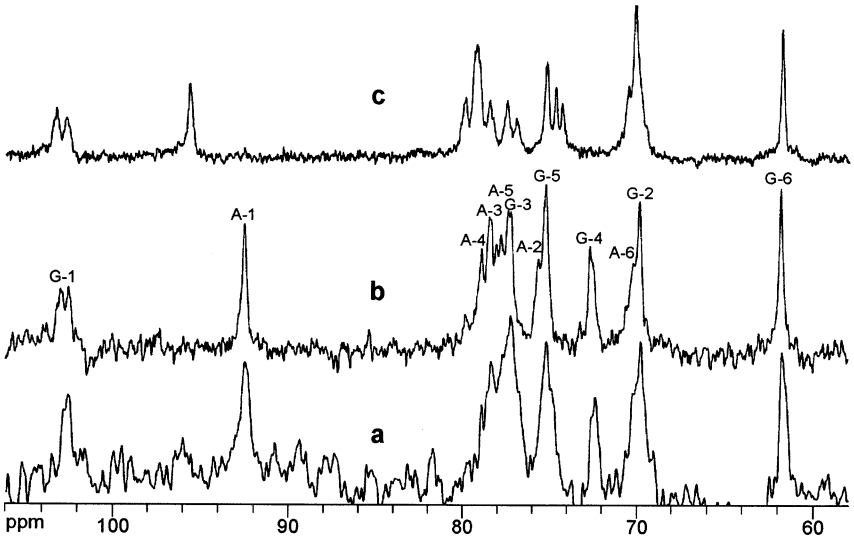

Fig. 3. ^{13}C NMR spectra of polysaccharides from (a) *Coccotylus truncatus* (5-h extraction in 0.1 M KOH solution) and commercial preparations (Sigma): (b) Iota carrageenan from *Eucheuma spinosa* and (c) Kappa carrageenan (for comparison).

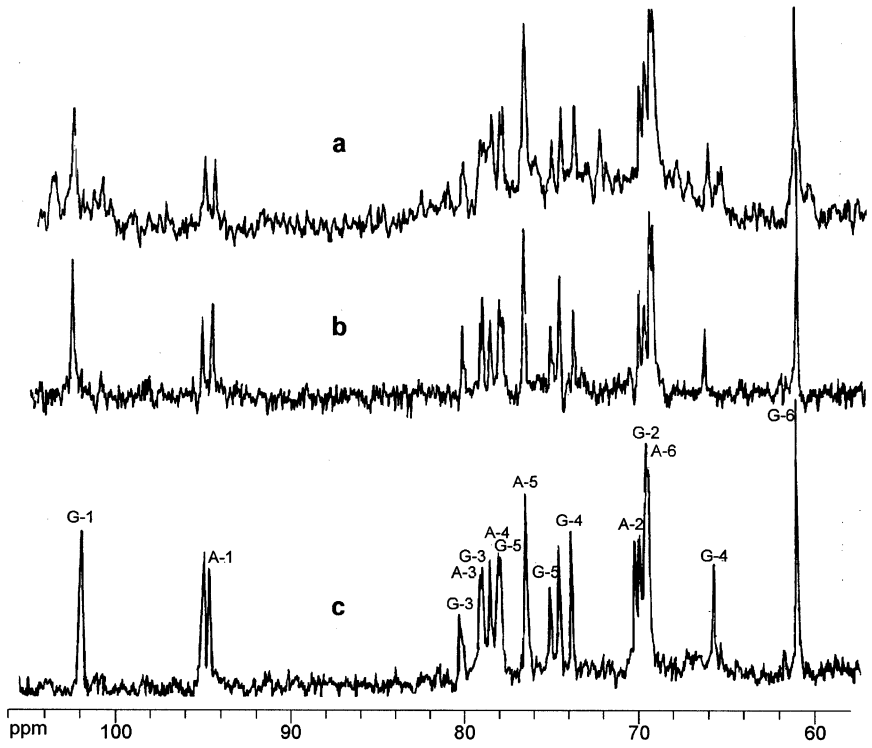

Fig. 4. ^{13}C NMR spectra of polysaccharides isolated from *Furcellaria lumbricalis* by 3-h extraction: (a) in water; (b) in water, kappa fraction; (c) in 0.16 M KOH solution.

(Fig. 4(c) and (b) respectively). Hence the effect of alkali on this furcellaran is comparable to fractionation of the best gelling component.

The spectra of these hypo-sulphated galactans are similar to those from *Furcellaria lumbricalis* of Norwegian waters [25].

3.4. Gelling ability

Galactans from *Coccotylus truncatus* form no gel under the conditions observed (the effect of calcium ions was not investigated in our study).

The great effect of potassium ions on the gelling ability of carrageenose 4′-sulphate is widely known. Fig. 5 illustrates this influence from the aspect of extracting/modifying medium (results on the basis of 4-h extraction).

As it is seen in Fig. 5(a), practical saturation with potassium ions is achieved already at low concentrations (≈ 0.08 M) of the extracting medium. The maximums of 3,6-anhydrogalactose content (Fig. 5(b)) and gel strength (Fig. 5(c)) do not coincide at the scale of KOH concentration. In this concrete research series, the maximum gel strength (670 g/cm^2) was achieved at the absolute maximum of possible potassium ion content (6.7% K^+) bound to the matrix. But broadly, the extraction of this algal species in KOH solution causes a 5-fold gel strength increase (in comparison with water-extracted product). Gelling properties, in addition to the above, depend greatly on the extraction time, algae location and season of collecting.

The extraction of galactans from *Furcellaria lumbricalis* for over 4 h is not advisable: gel strength decrease indicates the beginning of destruction of the polymer chain.

Simultaneous extraction of algae mixtures of *Furcellaria lumbricalis* and *Coccotylus truncatus* (in ratio 1:1 by mass) in 0.1 M KOH solution causes levelling of the gel strength of galactans from different fractions. Although iota car-

rageenan does not form a gel (in the conditions of our study), this mixture of seaweeds gave a product with an average gel strength 470 g/cm^2, forming 85–90% from the gel strength of *Furcellaria* galactans alone. The results are in accordance with general conceptions [6,7] about mixed gels of iota and kappa carrageenan and may be useful for practical applications. The gel strength of galactans extracted from this algae mixture (nearly corresponding to the proportion in the algal stratum in Kassari, the Baltic Sea) remains quite stable during the first 2.5 h of extraction.

4. Conclusions

Galactans of *Furcellaria lumbricalis* (Kassari Bay, the Baltic Sea) contain 6-*O*-methylgalactose and 2-*O*-methyl-3,6-anhydrogalactose in addition to the unsubstituted residues in their composition.

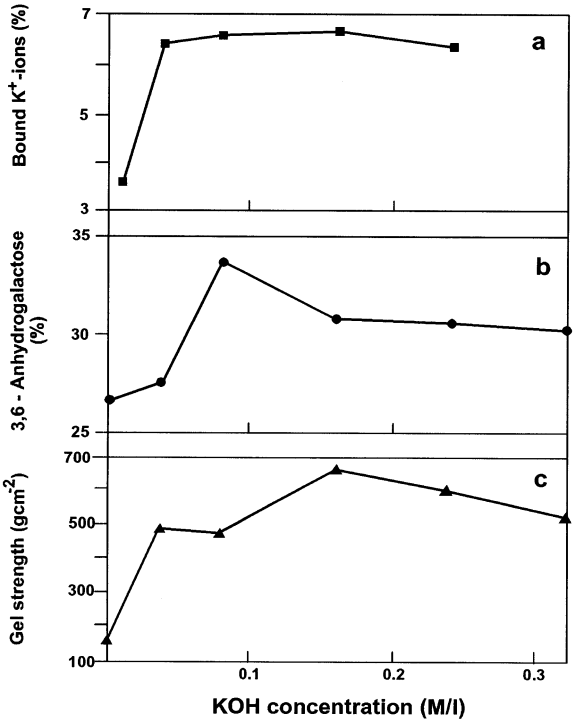

Fig. 5. Correlations between (a) potassium and (b) 3,6-anhydrogalactose content and (c) gel strength of the galactans extracted from *Furcellaria lumbricalis* at various KOH concentrations.

The gel strength of the native (water-extracted) galactans is low but may be considerably (about five times) increased by alkaline extraction of the seaweeds. From this standpoint, the most suitable medium for extraction with simultaneous modification of these algal galactans is potassium hydroxide solution at the concentration of 0.16 M. As a result of that treatment, the molar ratio 3,6-anhydrogalactose/galactose of the product rises from 0.69 to 0.82 and its structural pattern becomes close to those of the kappa fraction.

Another algal species *Coccotylus truncatus* occurring in Kassari Bay, has a thick epithelium inhibiting the water extraction of the seaweed. This process may be also promoted by alkali in solution causing no structural modification of the galactans. *Coccotylus truncatus* contains viscous galactans of iota carrageenan type, their structure is close to those from the species *Eucheuma spinosa* (a product of Sigma) and from *Phyllophora brodiaei* (the Black Sea).

The sulphur content of both galactan types is nearly half from the stoichiometric one. The galactan mixture formed by simultaneous alkaline extraction of both seaweeds has a high gel strength and can be used for applications in the food industry.

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