Proc. Estonian Acad. Sci. Chem., 1998, **47**, 2, 51–59
https://doi.org/10.3176/chem.1998.2.01

COMPOSITION AND STRUCTURE OF SUMMARY POLYSACCHARIDE FROM THE RED ALGA *Furcellaria lumbricalis*

Merike VAHER[a], Kalle TRUUS[a], Imants TAURE[b], Ivo LEITO[c], Lilja LAHE[a], Urve KALLAVUS[d], and Tõnis PEHK[e]

[a] Institute of Chemistry at Tallinn Technical University, Akadeemia tee 15, EE-0026 Tallinn, Estonia
[b] Centre for Nuclear Research, LV-2169 Salaspils, Latvia
[c] Institute of Chemical Physics, University of Tartu, Jakobi 2, EE-2400 Tartu, Estonia
[d] Centre for Materials Research, Tallinn Technical University, Ehitajate tee 5, EE-0026 Tallinn, Estonia
[e] Institute of Chemical and Biological Physics, Akadeemia tee 23, EE-0026 Tallinn, Estonia

Received 3 February 1998

Abstract. Summary polysaccharide (SP) of water extraction from *Furcellaria lumbricalis* is characterized by physical methods (^{13}C NMR and FTIR spectroscopy, neutron activation analysis). The polysaccharic and inorganic parts of SP were analysed. The composition of SP appears to be much more complicated than described earlier. Furcellaran cannot be divided into fractions (except the kappa fraction) on the basis of solubility in KCl solution; no iota fragments are present in this polysaccharide.

Key words: furcellaran, *Furcellaria lumbricalis*, kappa carrageenan, iota carrageenan.

INTRODUCTION

Summary polysaccharide (SP) of red algae is a product of the seaweeds extraction, isolated from the solution by alcohol precipitation. The principal component of this complex polysaccharide is specific sulphated galactans, so-called carrageenans in our case. Furcellaran is the traditional name for hyposulphated carrageenans from *Furcellaria lumbricalis*.

Two main types of carrageenans will be discussed – kappa and iota carrageenans (Fig. 1), or according to a new nomenclature [1], respectively, carrageenose 4´-sulphate and carrageenose 2,4´-disulphate. Various carrageenans

occurring in the algal biomass can often be separated from each other by the traditional precipitation process based on different solubility in potassium chloride solution [2, 3]. In the case of furcellaran at least, the full complex of SP cannot be interpreted as a mechanical mixture (it is shown in this paper). Data from the literature [4, 5] also refer to the presence of iota fragments in the galactan composition of the Baltic *F. lumbricalis* (former term: *Furcellaria fastigiata*). This issue was tested on the basis of IR spectra and KCl-fractionation in our work.

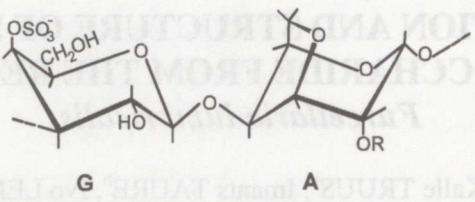


Fig. 1. Disaccharide repeating units of kappa (R = H) and iota ($R = SO_3^-$) carrageenan. G designates galactose and A anhydrogalactose residue.

Depending on their monosaccharide composition, algal galactans often have a gelling ability. SPs from various algae species have a wide practical use as gelling agents and thickeners in the food industry [6].

According to latest investigations [7], the monosaccharide composition of SP from *F. lumbricalis* is more complicated, including various methoxyl derivatives (up to 4.0% summarily from the SP mass) in addition to unsubstituted galactose and anhydrogalactose residues. Still, a certain generalization of the complex composition is necessary. Algal SP separated from the seaweed extract by alcohol precipitation has even more complicated composition as an inorganic part always occurs in real galactan preparations. This mineral component (and absorbed moisture) is the cause why SP from *F. lumbricalis* contains only 59–65% sugars [7]. The influence of inorganic cations on gelling processes is extremely important [8, 9] and this natural foil cannot be ignored (commercial carrageenan preparations for laboratory use contain usually more than 10% cations).

Although the carrageenan fractions have been widely investigated, the actual composition (and therefore, rheological properties) of algal polysaccharides has often remained unknown. Simple structural models are frequently insufficient for adequate description of real algal galactans [7].

Therefore, the purpose of the present study was to characterize the composition and structural features of the summary polysaccharide from whole water extract of *F. lumbricalis*.

MATERIAL AND METHODS

Algae samples. Seaweeds *Furcellaria lumbricalis* were collected from a depth of 6–8 m in Kassari Bay (the Baltic Sea, Estonia) by using an aqualung in July 1995. The plants were thoroughly washed in tap water and dried in air.

Extraction and precipitation. Suspension of algae in 30–50-fold mass of water or potassium hydroxide solution was refluxed above an air bath. The extract was filtered under rarefying through a porous glass filter (P2, Labklaas, Tallinn, Estonia). Algal polysaccharides were precipitated from the extract by adding it to ethanol (95% v/v, 3-fold volume per extract) and washed after filtration with ethanol (80% v/v).

Commercial samples. Carrageenan preparations were purchased from Sigma Chemical Co. 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 were objects of discussion in [10]. Commercial furcellaran preparation was a gift from Torben Hee, FMC A/S, Food Ingredients Division-Europe (Denmark).

Elementary composition (organogens). Carrageenan preparations were analysed using a Perkin Elmer PE 2400 CHNS (series 2) analyser (two parallels from air-dry samples).

Inorganic composition. The content of other elements was determined by neutron activation analysis. The samples were irradiated in a nuclear research reactor [11] of the Latvian Centre for Nuclear Research. A pneumatic tube conveyer was used to transport samples to and from the reactor.

For the determination of Mg, Al, Cl, Ti, V, Mn, Cu, and I the short-lived radionuclides were used. The samples were irradiated in horizontal channels at a flux of 1.6×10^{13} $ncm^{-2}s^{-1}$ for 30 s. The gamma-spectra from the irradiated samples were measured using a Ge (Li) detector connected to a 4000 channels analyser (NTA-1024, Hungary).

When long-lived nuclides were used for analysis, the irradiation of samples was performed in vertical channels at two regimes: with reactor neutrons at a flux of 1.4×10^{13} $ncm^{-2}s^{-1}$ for 24 h, and with resonance neutrons at a flux of 1.3×10^{11} $ncm^{-2}s^{-1}$ for 72 h. In both cases, the irradiated samples were measured twice: 4 days and 3 weeks after irradiation. The gamma-activity initiated was measured using a Ge (Li) detector connected to a 4000 channels analyser (LP-4900, Nokia, Finland). A more detailed description of the method is given in our preceding paper [12].

Scanning electron microscopy. The samples of air-dry algae were immersed in liquid nitrogen, cryofracturated, and dried in air. Then the 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 JSM 840 A). The electronic images were directly saved as TIF files in a personal computer.

Infrared spectroscopy. FTIR spectra of the carrageenan samples were scanned with an Interspec 2020 spectrometer from thin (0.015 mm) films obtained by slow evaporation of 1% solutions on the surface of mercury.

NMR spectroscopy. Proton-decoupled ^{13}C NMR spectra were measured on a Bruker AMX-500 spectrometer for 1–1.5% carrageenan solutions at 65°C. 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 a constant value (61.3 ppm) in these carrageenans [13].

RESULTS AND DISCUSSION

One of the main interests in this work was to elucidate the extent of heterogeneity of real polysaccharide preparations. SP consists of the following components: (1) the specific, main polysaccharic component, (2) ash component (inorganic part), (3) absorbed moisture, (4) non-specific polysaccharic component (cellulose and xylans from cell walls), (5) residues of algal pigments, and (6) processing contaminations.

The rheological properties of gelling galactans, causing their use, depend notably on the content of the components listed. This side of the 'structure–function' problem is unsatisfactorily revealed in the literature [7].

Table 1 demonstrates the SP heterogeneity from the viewpoint of organogenic composition.

Table 1

Elementary composition of some carrageenans (organogens)

Sample No.	Preparation, origin	Content of elements, %		
		C	H	N
1	SP from *Furcellaria lumbricalis* (extracted by 0.1 M KOH solution)	30.56	5.02	0.51
2	Commercial furcellaran (FMC A/S, Denmark)	29.17	4.82	0.10
3	Kappa carrageenan (Sigma)	26.76	4.58	0.09
4	Iota carrageenan (Sigma)	22.54	4.10	0.12

This nitrogen content of the samples is somewhat surprising and expresses quite a high level of algal proteinic pigments still occurring in actual galactan preparations. According to their carbon content, the samples presented in Table 1 contain only 60–70% of the basic substance, which agrees with earlier results [7].

The mineral part of SP from *F. lumbricalis* (Table 2) is especially rich in potassium, magnesium, and calcium. Enrichment of furcellaran with potassium is

a result of processing (extraction with potassium hydroxide solution); Mg and Ca originate from seawater. In case of some elements, a considerable purification can be observed (from algae to SP): for Mg 19.4 times, I 37.5 times, Br 32.8 times, etc. It is of interest to compare the purification effect with the case of less charged galactan matrices [12] where various elements are removed from the polysaccharide chain much more effectively. A certain enrichment of furcellaran with several heavy metals (Cr, Fe, Co, Zn) may be connected with the extraction vessel made of stainless steel or with the water-pipes coated with zinc.

Table 2

Composition of the inorganic part of *Furcellaria lumbricalis* and furcellaran extracted from the algae*

Element**	Element content, ppm		Element**	Element content, ppm	
	algae	furcellaran		algae	furcellaran
Sodium	400	500	Gold	< 0.11	0.042
Magnesium	88 300	4 550	Uranium	19.3	0.6
Aluminium	680	1 165	Selenium	0.42	< 0.069
Chlorine	110	< 200	Mercury	0.011	0.009
Potassium	<< 12 000	46 000	Chromium	2.5	3.4
Calcium	<< 8 000	< 1 300	Barium	< 33	50.5
Titanium	< 200	< 220	Strontium	280	< 20
Vanadium	2.3	< 0.6	Antimony	< 11	
Manganese	420	245	Cesium	0.093	0.18
Copper	33	< 15	Nickel	19.5	
Arsenic	< 25	98.5	Rubidium	11	22.5
Bromine	1 970	60	Iron	870	1 265
Molybdenum	1.7	< 0.09	Zinc	110	130
Iodine	413	11	Cobalt	1.5	1.65

* in 0.1 M KOH solution;
** in order of increasing atomic number.

Carrageenans occur mostly in cell walls of algae [14, 15]. There are also specific cylindrical cells which consist almost entirely of SP (Fig. 2) in *F. lumbricalis*. Thus, from the standpoint of ionic influence to galactan gels, algae live in very complicated physico-chemical conditions. Regardless of many excellent investigations (overviews [16, 17]) about the effects of cations on the gelation of carrageenans, actual processes in algal cell walls have not been sufficiently studied.

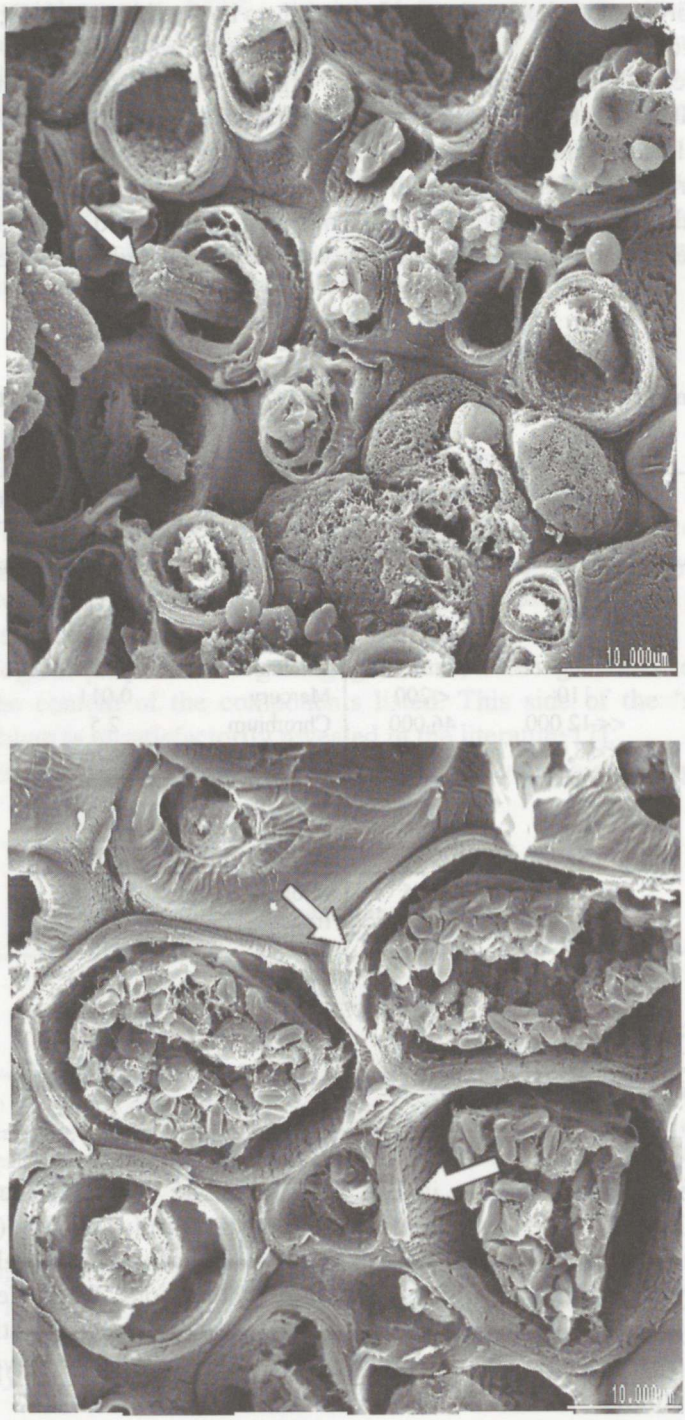
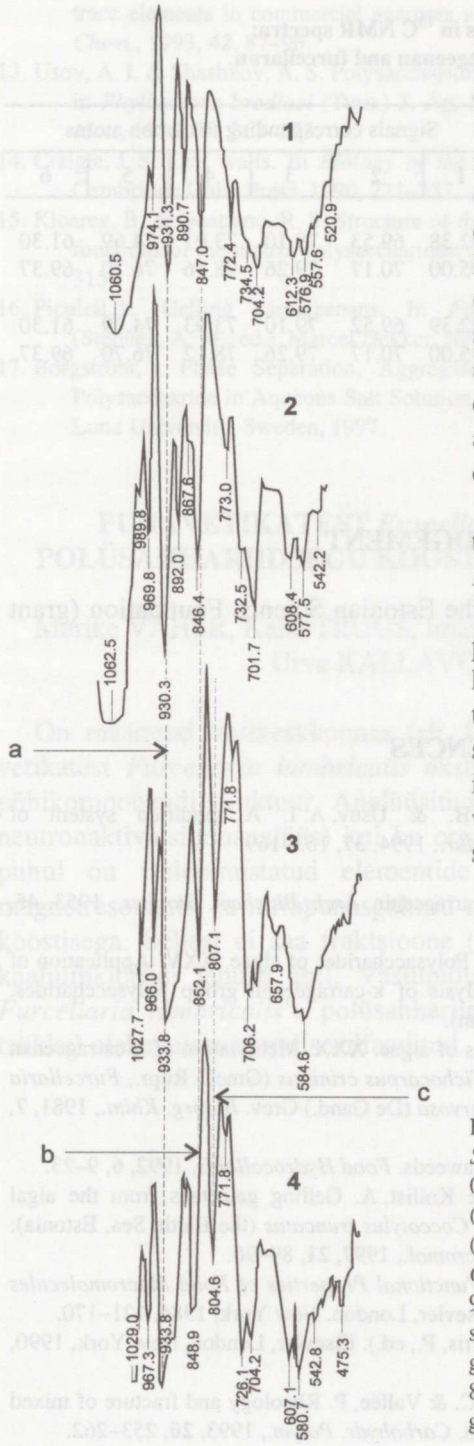


Fig. 2. Scanning electron micrographs of *Furcellaria lumbricalis* (cross-sections). Arrows indicate main location sites of galactans in algal cells.



Although minor components of furcellaran are very important for alkaline modification of SP [7], the main structural feature of this polysaccharide is connected with kappa carrageenan (Fig. 3). The infrared spectra prove, contrary to earlier results [4, 5], also total lack of iota fragments in furcellaran (both in laboratory and commercial samples). According to NMR data, the signals of 12 carbon atoms from the disaccharide unit of kappa carrageenan and laboratory furcellaran nearly coincide (Table 3).

Contrary to SP of water extraction, the alkali-extracted product from *F. lumbricalis* has a clear spectrum of ^{13}C NMR. Traditional fractionation procedures [2] cannot separate any homogeneous fraction from SP, except the kappa fraction. The last may be separated from SP on the basis of insolubility in 0.33 M KCl solution as well as by alkaline extraction of the seaweeds.

Fig. 3. FTIR spectra of carrageenan preparations. *Samples*: 1, laboratory furcellaran extracted by 0.1 M KOH solution; 2, commercial furcellaran (FMC A/S, Denmark); 3, iota carrageenan (Sigma); 4, laboratory iota carrageenan from *Coccotylus truncatus*. *Specific regions*: a, 930–934 cm^{-1} (3,6-anhydrogalactose, present in all samples); b, 846–852 cm^{-1} (4-sulphate groups of galactose residues, in all samples); c, 805–810 cm^{-1} (2-sulphate groups of 3,6-anhydrogalactose residues, only in samples 3, 4).

Table 3

Assignment of resonances in ^{13}C NMR spectra: comparison of kappa carrageenan and furcellaran

Polysaccharide	Residue*	Signals corresponding to carbon atoms					
		1	2	3	4	5	6
κ-Carrageenan (commercial)	G	102.38	69.53	79.10	73.91	74.69	61.30
	A	95.00	70.17	79.26	78.16	76.70	69.37
Furcellaran (SP of alkaline extraction)	G	102.39	69.52	79.10	73.93	74.69	61.30
	A	95.00	70.17	79.26	78.12	76.70	69.37

* see Fig. 1.

ACKNOWLEDGEMENT

The work was partially supported by the Estonian Science Foundation (grant No. 2860).

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PUNAVETIKATEST *Furcellaria lumbricalis* ERALDATUD POLÜSAHHARIIDSEGU KOOSTIS JA STRUKTUURI PÕHITÜÜP

Merike VAHER, Kalle TRUUS, Imants TAURE, Ivo LEITO, Lilja LAHE, Urve KALLAVUS ja Tõnis PEHK

On määratud vesikeskkonnas (sh. leelise lisandi korral) Läänemere punavetikatest *Furcellaria lumbricalis* ekstraheeritud polüsahhariidsegu koostis ja põhikomponendi struktuur. Analüüsitud on nii segu anorgaanilist (28 elementi, neutronaktivatsioonanalüüs) kui ka orgaanilist koostist. Anorgaanilise koostise puhul on iseloomustatud elementide ekstraktsioonidünaamikat. ^{13}C-tuuma-magnetresonants- ja infrapunaspektrid näitavad, et orgaaniline osa on keerulise koostisega. Sellest ei saa fraktsioone (välja arvatud kapa-fraktsioon) eraldada kaaliumkloriidi lahuse abil. Vastupidiselt varasematele andmetele ei sisalda *Furcellaria lumbricalis*'e polüsahhariidid nn. ioota-fragmente, kus galaktoos-tsükkel oleks teises asendis sulfaaditud.