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Carrageenans from cystocarpic and sterile plants of *Chondrus pinnulatus* (Gigartinaceae, Rhodophyta) collected from the Russian Pacific coast

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Abstract

The chemical structure, gel properties and biological activity of the carrageenans isolated from cystocarpic and sterile plants of *Chondrus pinnulatus* were investigated. The total carrageenan content of the sterile plant was observed to be twice that of the cystocarpic plants. According to data obtained by ^{13}C-NMR and FT IR, the gelling polysaccharides from cystocarpic and sterile plants of *C. pinulatus* have similar structures and were identified as κ/ι-carrageenans. The difference between these polysaccharides was in the ratio of the κ- and ι-segments, with a predominant content of κ-segments in cystocarpic plants (80%). Moreover, KCl-insoluble fractions possibly contain hetero-disperse μ/ν precursor: amounts of this in the polysaccharide from sterile plants were more than that extracted from the cystocarpic plants. The KCl-soluble fractions (non gelling) were λ-carrageenans with another carrageenan type that had a low amount of 3,6-anhydrogalactose. Carrageenans from cystocarpic stages showed good gelling properties, whereas those from sterile plants formed a very weak gel. Structural differences and molecular weight of carrageenans obviously determine the biological activity of the polysaccharides. Non gelling-carrageenans from both types of *C. pinnulatus* plants showed high macrophage-phosphatase activity and κ/ι-carrageenan from cystocarpic plant possessed a potent anti-coagulant activity, which was extremely strong in a low concentration of 100 μg mL^{-1}.

Introduction

Carrageenans are a complex family of water-soluble galactans extracted from marine red algae that have applications as gelling, thickening and suspending agents in food processing. Carrageenans have pronounced biological activities or other properties useful in the biomedical field. These polysaccharides are composed of alternating α-(1–3) and β-(1–4) linked D-galactosyl residues and several types of carrageenan are identified on the basis of the modification of the disaccharide repeating unit by sulphate esters and by the presence of 3,6 -anhydrogalactose as 4-linked residue. At least 16 types of carrageenans have been defined, some of which have no commercial importance so far (Knutsen et al., 1994). Native carrageenans are often hybrids of more than one type of repeating unit (Craigie, 1990; Knutsen et al., 1994). Variations in carrageenan structures are known to occur not only between different species of the Gigartinaceae, but also between different life stages of the same species (McCandless et al., 1973; Craigie, 1990; Stortz & Cerezo, 1993; Falshaw & Furneaux, 1994). It has been shown that members of the family Gigartinaceae yield different carrageenans from karyologically different generations (Craigie, 1990). Close studies of the carrageenans from cystocarpic and tetrasporic plants of *Iridaea undulosa* (Stortz & Cerezo, 1993) and *Gigartina skottsbergii* (Matulewicz et al., 1989) showed that the cystocarpic plants exhibit two major products

separable by potassium chloride precipitation: one of the products is a soluble, partially cyclisized μ/ν- carrageenan and the other is a gelling κ/ι-carrageenan. The tetrasporic plants produce sets of λ-carrageenans gelling at high concentrations of potassium chloride. The polysaccharides that have been extracted from tetrasporic plants of *Gigartina clavifera* and *G. alveata* are predominantly ξ-carrageenans (Falshaw & Furneaux, 1995). This suggests the taxonomic position of algae does not provide full information on the type of polysaccharide they contain. Therefore, the establishment of the polysaccharide composition of an alga in relation to the phase of its life cycle remains an important research objective.

Carrageenans have a wide spectrum of biological action (Lahaye & Kaeffer, 1997; Yermak & Khotimchenko, 2003), depending on the polysaccharide structure (Güven et al., 1990). *Chondrus pinnulatus* is abundant in the Russian Far-Eastern seas. This species of marine alga grow mostly on lower eulittoral and upper sublittoral rocks and stones, on open coasts with low wave action. The system of carrageenans from *C. pinnulatu*s has been studied with material extracted from unsorted (mixed phase) samples (Yermak et al., 1999). The aim of the present study was to investigate the structure, gel properties and biological activity of the systems of carrageenans isolated from cystocarpic and sterile plants of *C. pinnulatus* (Gigartinaceae) collected from the Great Peter Bay (the Sea of Japan).

Materials and methods

Algae

Chondrus pinnulatus (Harv.) Okam. is widespread in the Sea of Japan. Material was harvested in The Great Peter Bay at the end of August and separated into sterile plants (SP) and plants with cystocarps (CP).

Extraction

Dried and milled algae (50 g) were suspended in hot water (1.5 L) and the polysaccharides were extracted at 90 °C for 2 h in a boiling water bath. The suspensions were centrifuged (2 500 g, 20 min, 20 °C) and the algal residues were re-extracted twice with water for 2 h in a boiling water bath. The supernatants were pooled. The polysaccharides were separated into the gelling-KCl-insoluble (a) and non-gelling the KCl-soluble fractions (b) as described previously (Yermak et al., 1999).

Analytical methods

The total amount of carbohydrates was estimated using the phenol-sulphuric acid method, using D-galactose as standard (Dubois et al., 1956). Monosaccharides as alditols acetate derivatives (Englyst & Cummings, 1984) were identified by GLC using an Agilent 6850 gas chromatograph equipped with a HP-5MS capillary column (30 m × 0.25 mm) with 5% Phenyl Methyl Siloxane and flame-ionization detector. The analyses were carried out at temperature programming from 175 to 220 °C with 3 °C min^{-1}. The content of 3,6-anhydrogalactose was determined according to the method of Usov and Elashvili (1991). The protein content of samples was determined according to the method of Lowry et al. (1951) using crystalline bovine serum albumin as the standard protein. The content of ash in the polysaccharides was determined gravimetrically after incineration of samples at 550 °C for 16 h followed by 2 h at 900 °C. The sulphate ester content of polysaccharide was determined according to the method Lahaye and Axelos (1993) by HPLC equipped (conductivity detector Waters 431) with a IC-Park A Anion column (50 × 4.6 mm 10 μm, Waters), eluted by 2 mM borate/gluconate eluent (flow rate: 1.0 mL min^{-1}).

Infrared spectroscopy and nuclear magnetic resonance spectroscopy

Films of polysaccharides for infrared analysis were obtained by drying in polyethylene molds (about 0.5 cm deep, 2.5 cm diameter) at 35–40 °C, 2 mL of an aqueous solution containing 5–7 mg of polysaccharide. The polysaccharide film was clamped between NaCl windows and the IR spectrum was recorded in the 4000–600 cm^{-1} region using a Bruker Vector 22 instrument, taking 240 scans with a resolution of 2 cm^{-1}. ^{13}C-NMR spectra of polysaccharide solutions in D_2O were recorded with a DXR-500 spectrometer operating at 60 °C and 62.9 MHz. Chemical shifts were determined from CD_3OD assigned at 50.15 ppm and used as an internal standard.

Sedimentation

The molecular weights of carrageenan solutions (0.1% w/v) in 0.1 mol L^{-1} NaCl were determined using an