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Characterization of a fucoidan from *Lessonia vadosa* (Phaeophyta) and its anticoagulant and elicitor properties

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Abstract

Blades of *Lessonia vadosa* (Phaeophyta) were extracted with 2% $CaCl_2$ solution, affording in 4.4% yield a polysaccharide which contained fucose and sulfate groups in the molar ratio 1.0:1.12. The high negative optical activity value ($[\alpha]_D^{22} = -134.0^\circ$), FT-IR and NMR analysis suggest the presence of a fucoidan. ^{13}C NMR spectrum of the polysaccharide obtained by solvolytic desulfation of native fucoidan indicated the major presence of 1 → 3 linked α-L-fucan. Depolymerization of the native fucoidan with H_2O_2 in the presence of copper(II) acetate gave in 54.8% yield a fraction with 33.7% of sulfate content. The native fucoidan (MW 320,000) showed good anticoagulant activity whereas the radical depolymerized fraction (MW 32,000) presented a weak anticoagulant activity. These polysaccharides showed significant activation of phenylalanine-ammonia lyase (PAL), lipooxygenase (LOX) and glutathione-*S*-transferase (GST) defence enzyme activities in tobacco plants.

Keywords: *Lessonia vadosa*; Phaeophyceae; Fucoidan; Anticoagulant; Elicitor

1. Introduction

Many species of brown seaweeds (Phaeophyceae) produce sulfated fucans. Besides L-fucose, they frequently contain D-xylose, D-galactose, D-mannose and D-glucuronic acid [1,2]. The biological activities of sulfated fucans have been extensively examined but their structures were scarcely well established. These polysaccharides are heterogeneous and branched and they may contain, besides additional monosaccharides, acetyl groups [3,4]. Fucoidans are algal sulfated polymers containing substantial amounts of L-fucose and sulfate groups [1,5]. According to Patankar et al. [6], their structure corresponds to a branched α-1 → 3-linked L-fucose polymer mainly sulfated at position *O*-4. Chevolot et al. [7,8] obtained by partial hydrolysis of the complex family of fucans from seaweeds such as *Ascophyllum nodosum* and *Fucus vesiculosus* fucoidan fractions with a backbone of α-(1 → 3) and α(1 → 4) linkages with sulfation at positions *O*-2 and *O*-2,3. Bilan et al. [9] informed that a fraction of the fucoidan from *Fucus distichus* was built up of alternating 3-linked-α-L-fucopyranose 2,4disulfate and 4-linked-α-L-fucopyranose 2-sulfate residues.

Species of the genus *Lessonia* of the Lessoniaceae family (Laminariales) grow abundantly in the Chilean cost [10]. Percival et al. [11] by sequential extraction of *Lessonia nigrescens* obtained fucans with low content of sulfate groups (6–13%) and with considerable amounts of uronic acid (9–29%). On the other hand, *Lessonia flavicans* afforded a fucan devoid of uronic acid with 35.5% of sulfate groups [12]. Extraction with acid of *Lessonia trabeculata* gave a mixture of fucans, which was fractionated by ion-exchange chromatography. It was found that as the ionic strength of the eluant increases, the amount of uronic acids decreases with increase in the content of sulfate groups [13]. Recently, by sequential extraction of *Lessonia vadosa* blades, fractions containing sulfated fucans were obtained [14].

Sulfated fucans have been known to possess many biological activities such as anticoagulant, antiviral, anti-inflammatory and prevent cell proliferation and adhesion [2–4,15].

Likewise, it has been reported that seaweed polysaccharides can act as elicitors of plant defence. Mercier et al. [16] evaluated the effects of laminaran and carrageenans on defence responses and signalling in tobacco plants. They found

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that only carrageenans induced signalling and defence gene expression in tobacco leaves. An elicitor oligosaccharide was obtained by enzymatic hydrolysis of laminaran from the brown seaweed *Eisenia bicyclis* [17]. Kupper et al. [18] studied the elicitor activity of the depolymerized products of alginic acid in sporophytes of *Laminaria digitata*, they found that oligoguluronate is the most active fraction to elicit an oxidative burst. The elicitor activity of the polymannuronic acid enriched fraction from alginic acid of blades of *L. vadosa* was assayed in wheat plants. It was found that the polymannuronic acid enriched fraction induced substantial elicitation of phenylalanine-ammonia-lyase and peroxidase activities [19]. Klarzynski et al. [20] obtained oligosaccharides by enzymic hydrolysis of the sulfated fucan from *Pelvetia canaliculata* which showed potent elicitor activity in tobacco plants. Recently, fractions of polymannuronic acid from *L. vadosa* and of sulfated galactan from *Schizymenia binderi* (Rhodophyta) showed to stimulate growth and defence in tobacco plants [21].

This work is devoted to the characterization of the fucan obtained by direct extraction of *L. vadosa* (Laminariales, Phaeophyta), the preparation of a low molecular weight fraction by its partial depolymerization and the study of their anticoagulant property and elicitor activities in tobacco plants.

2. Experimental

2.1. Materials and methods

L. vadosa Searles was collected during autumn in north Fuerte Bulnes (53°37′54.7″S, 70°55′17.4″W). Specimens were deposited in Sala de Colecciones, Departamento de Ciencias y Recursos Naturales, Universidad de Magallanes, Punta Arenas, Chile.

FT-IR spectra in KBr pellets were registered in the 4000–400 cm^{-1} region using a Bruker IFS 66*v* instrument according to Cáceres et al. [22]. ^{1}H NMR (400.13 MHz) and ^{13}C (100.62 MHz) spectra of the polysaccharides were recorded in D_2O at 70 °C on a Bruker Avance DRX400 spectrometer using internal methanol ($\delta^{13}C$ 49.50 ppm, $\delta^{1}H$ 3.340) as reference. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. Microanalysis was performed at Facultad de Química, Pontificia Universidad Católica de Chile. Total sugars were determined by the phenol–sulfuric acid method [23]. Molecular weight was determined by the reducing end method as previously described [24]. Sulfate content was analysed by the turbidimetric method of Dodgson and Price [25] and as SO_2 with IR S-1444 DR LECO Analyzer as described by Escalona et al. [26]. The content of uronic acid was determined following the method of Filisetti-Cozzi and Carpita [27] using D-galacturonic acid as standard. Gas–liquid chromatography (GLC) was carried out on a Shimadzu GC-14B chromatograph equipped with a flame ionisation detector using a SP 2330 column (0.25 mm × 30 m) and performed with an initial 5 min hold at 150 °C and then at 5 °C min^{-1} to 210 °C for 10 min. The helium flow was 20 mL min^{-1}.

2.2. Extraction

Blades of *L. vadosa* were oven dried at 50 °C for 36 h. The dry seaweed (120 g) was milled and stirred for 10 min with 1 L of petroleum ether (b.p. 40–60 °C). The supernatant was concentrated in vacuo and the extraction process was repeated until no more solid residue was obtained (three times) in the concentrate. The residual petroleum ether was evaporated at room temperature for 72 h and the algae was treated with 1.6 L of 96% ethanol and 0.4 L of 37% aqueous formaldehyde for 72 h and air-dried. One hundred grams of the dried algae were extracted with 500 mL of 2% aqueous calcium chloride solution for 5 h at 85 °C. The supernatant was separated by centrifugation (4000 × *g*) and dialysed (3500 Da cut-off membrane) against tap water, followed by distilled water, concentrated in vacuo and freeze-dried. The resulting solid was dissolved in 150 mL of distilled water, stirred for 2 h with 1 M HCl (50 mL) and centrifuged. The supernatant was neutralized with 1 M NaOH, dialysed against distilled water, concentrated and freeze-dried.

2.3. Hydrolysis

The polysaccharide (0.003 g) was heated with 0.5 mL of 2 M trifluoroacetic acid for 2 h at 120 °C. The acid was removed in vacuo by co-evaporations with distilled water and the resulting syrup was treated with sodium borohydride in 3 mL of distilled water. The reduced material was acetylated with acetic anhydride in dry pyridine and analysed by GLC. Acetates of L-fucitol, D-glucitol, D-mannitol, D-galactitol, L-rhamnitol and D-xylitol were used as standards.

2.4. Desulfation

The polysaccharide (0.075 g) was dissolved in 7.5 mL of anhydrous pyridine and 3.7 mL of chlotrimethylsilane were added [28]. The solution was refluxed for 8 h at 100 °C with stirring. After cooling, the excess of chlorotrimethylsilane was destroyed by dropwise addition of water at 0 °C. The resulting solution was dialysed against tap water, followed by distilled water, 0.1 M NaCl solution, distilled water and freeze-dried.

2.5. Free radical depolymerization

The polysaccharide (1.00 g) and copper(II) acetate monohydrate (0.160 g) were dissolved in 60 mL of distilled water at 60 °C. Then, 36 mL of 9% H_2O_2 solution was added at 12 mL/h at 60 °C and the pH of the solution was maintained at 7.5 by addition of 2 M NaOH solution [29]. The reaction mixture was treated with Chelex 100 resin (sodium salt) to remove Cu^{2+} ions, dialysed extensively against distilled water using a 3500 Da cut-off membrane and freeze-dried.

2.6. Gel permeation chromatography

The polysaccharide (2 mL of 0.003 g/mL solution) was chromatographed on a Sephadex G-200 column (100 cm × 1.5 cm) using 0.4 M NaCl as eluant. The column was calibrated with