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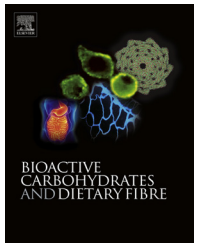

Bioactive polysaccharides from marine algae

Tamara Barahona[a], *María V. Encinas*[a], *Mónica Imarai*[b], *Andrés Mansilla*[c], *Betty Matsuhiro*[a,*], *Rodrigo Torres*[a], *Beatriz Valenzuela*[b]

[a]*Departamento de Ciencias del Ambiente, Santiago, Chile*
[b]*Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile, Av. L. B. O'Higgins 3363, Santiago, Chile*
[c]*Departamento de Ciencias y Recursos Naturales, Facultad de Ciencias, Universidad de Magallanes, Av. Bulnes 1465, Punta Arenas, Chile*

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ABSTRACT

Laminaran, fucoidan and sulfated galactan were extracted from the seaweeds *Desmarestia distans*, *Lessonia vadosa*, and *Gigartina skottsbergii*, respectively. Modified polysaccharides were prepared by desulfation, depolymerization, and sulfation. Antioxidant, anticoagulant and immunostimulating activities of native and modified polysaccharides were assayed *in vitro*. The oversulfated derivatives showed high antioxidant capacity towards oxygen radical assay; however, no direct relation between sulfate content and antioxidant capacity was found. Oversulfated polysaccharides presented higher antioxidant capacity towards hydroxyl radicals than the native polysaccharide. Regarding the $ABTS^{\bullet+}$radical cation assay moderate inhibition values (35.1–3.4%) were observed. The anticoagulant activity of native and modified polysaccharides was measured using the activated partial thromboplastin time assay; the native sulfated galactan from *G. skottsbergii* presented the highest value, close to that shown by heparin at similar concentrations. The immunostimulating activity of polysaccharides was measured through their effects on bone marrow-derived mice dendritic cell maturation. The native sulfated galactan from *G. skottsbergii* presented good dose-dependent activity inducing increased levels of MHC class II in dendritic cells.

1. Introduction

In the last decades the study of polysaccharides from marine algae has gained renewed interest for their many valuable biological properties (Pomin & Mourão, 2010; Wijesekara, Pangestuti, & Kim, 2011). Brown seaweeds (Phaeophyceae) mainly produce alginic acid, and to lesser extent fucans and laminarans. Fucans, the polymers of L-fucose, may also contain galactose, mannose, xylose and uronic acids and sulfate groups; the term fucoidan is restricted to sulfated homo-L-fucans (Patankar, Oehninger, Barnett, Williams, & Clark, 1993; Chevolot, Mulloy, Ratiskol, Foucault, & Colliec-Jouault, 2001; Bilan et al., 2002). Fucoidans have received much attention due to their many biological activities including, anticoagulant, antithrombotic, antitumor, antioxidant, antiviral and anti-inflammatory properties (Berteau & Mulloy, 2003; Li, Lu, Wei, & Zhao, 2008). Recently, commercial fucoidan had shown immunomodulatory properties on human and mice dendritic cells (Kim & Joo, 2008; Yang et al., 2008b). Laminarans are neutral 1→3-beta-D-glucans

*Corresponding author. Tel.: +56 2 27181159.
E-mail address: betty.matsuhiro@usach.cl (B. Matsuhiro).

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with beta-1,6 branching (Painter, 1983; Rioux, Turgeon, & Beaulieu, 2010). Lai et al. (2010) studied the immunomodulatory and adjuvant activities of a high molecular weight 1→3-beta-D-glucan fraction isolated from *Ganoderma lucidum*. They found that in mice this glucan showed an increase in the number of dendritic cells and displayed potent adjuvant activity while *in vitro* it induced the maturation of dendritic cells and stimulated the production of cytokines and chemokines. Agarans and carrageenans are the most common sulfated galactans from red seaweeds; they differ in the configuration of alfa-galactopyranosyl residues and in the pattern of sulfation (Lahaye, 2001; Van de Velde, Knutsen, Usov, Rollema, & Cerezo, 2002; Campo, Kawano, Braz da Silva, & Carvalho, 2009). Various sulfated galactans have been studied in search of a relation between sulfate presence and anticoagulant activity (Farías, Valente, Pereira, & Mourão, 2000; Pereira, Melo, & Mourão, 2002; Pereira et al., 2005; Fonseca et al., 2008). It seems that there is no correlation between coagulation with sulfate content. However, Opoku, Qiu, and Doctor (2006) found that oversulfation of kappa-carrageenan gave a derivative with 30 times higher anticoagulant activity than the native carrageenan. Moreover, it has been reported that sulfated polysaccharides from seaweeds showed *in vitro* antioxidant capacity (Ruperez, Ahrazem, & Leal, 2002; Kim et al., 2007; Rocha de Souza et al., 2007; Barahona, Chandía, Encinas, Matsuhiro, & Zúñiga, 2011). Recently, Gómez-Ordóñez, Jiménez-Escrig, and Rupérez (2014) reported the antioxidant and anticoagulant activities of kappa-iota hybrid carrageenans from *Mastocarpus stellatus* (Rhodophyta). The brown seaweeds *Desmarestia distans* and *Lessonia vadosa*, and the green variant of tetrasporic *Gigartina skottsbergii* (Rhodophyta) grow abundantly in southern Chile, but so far they are not exploited for industrial purposes. The aim of this work is the study of antioxidant, anticoagulant, and immunostimulating properties of polysaccharides extracted from these polysaccharides. In order to study the relationship among sulfate content, molecular weight and biological activity, native polysaccharides were modified by oversulfation, desulfation and partial hydrolysis.

2. Materials and methods

2.1. Materials and general methods

D. distans (C. Agardh) J. Agardh, *L. vadosa* Searles, and the green variant of tetrasporic *G. skottsbergii* Setchellet Gardner were collected in Fuerte Bulnes (53°37′55 6″S, 70°55′17 9″W), Magellan region. Specimens were deposited in Sala de Colecciones, Departamento de Ciencias y Recursos Naturales, Universidad de Magallanes, Punta Arenas, Chile. Preparation of fucoidan from *L. vadosa* was performed according to Chandía and Matsuhiro (2008). Isolation of sulfated galactans from the green variant of tetrasporic *G. skottsbergii* and their chemical modifications were previously described (Barahona, Encinas, Mansilla, Matsuhiro, & Zúñiga, 2012). FT-IR spectra in KBr pellets were registered in the 4000–400 cm^{-1} region using a Bruker IFS 66*v* instrument (Bruker, Coventry, UK) according to Leal, Matsuhiro, Rossi, and Caruso (2008). Absorbance was measured in a Genesys 5 Thermospectronic spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). ^{1}H NMR (400.13 MHz) and ^{13}C (100.62 MHz) spectra of the polysaccharides were recorded in D_2O, after isotopic exchange (3 × 0.75 mL) at 70 °C on a Bruker Avance DRX 400 spectrometer (Bruker, Coventry, UK) using the sodium salt of 3-(trimethylsilyl)-1-propane-d_4-sulfonic acid) as internal reference. Gas–liquid chromatography (GC) was carried out on a Shimadzu GC-14B chromatograph (Shimadzu, Tokyo, Japan) equipped with a flame ionization 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 to 210 °C for 10 min. The helium flow was 20 mL/min. Molecular weight of polysaccharides was determined by the reducing end method (Park & Johnson, 1949; Cáceres, Carlucci, Damonte, Matsuhiro, & Zúñiga, 2000). Reagent grade solvents were purchased from Merck (Darmstadt, Germany); DEAE-Sephadex, Sephadex 100 and reagent grade chemicals were purchased from Sigma (St. Louis, MO, USA).

2.2. Extraction of Desmarestia distans

Blades of *D. distans* were oven dried at 45 °C for 48 h. The dry seaweed (120 g) was milled and stirred for 30 min with 900 mL of *n*-hexane. The supernatant was concentrated *in vacuo* and the treatment was repeated four times. The treated seaweed was dried at room temperature for 48 h and soaked into 2000 mL of a solution containing 96% ethanol and 36% of formaldehyde in a 4:1 v/v ratio. After 72 h the seaweed was decanted and air dried. One hundred grams of the dried alga were stirred with 3% $CaCl_2$ solution at 85 °C for 4 h, and the mixture was cooled and centrifuged at 3000 × *g* for 20 min. The solid was recovered and the extraction process was repeated three more times. The supernatants were dialyzed using Spectra/Por membrane (MWCO 3500) (Spectrum Laboratories, Rancho Domínguez, CA, USA) against tap water, followed by distilled water, concentrated *in vacuo* and freeze-dried (Christ Alpha 1–2 Freeze Dryer, Osterode am Harz, Germany). The resulting solid was dissolved in 75 mL of distilled water, stirred for 2 h with 1 M HCl (50 mL) and centrifuged. The supernatant was neutralized with 1 M NaOH solution, dialyzed against distilled water, concentrated and freeze-dried.

2.3. Ion-exchange chromatography

The $CaCl_2$ extract from *D. distans* was dissolved in water (0.2 g/mL solution) and was deposited on a DEAE-Sephadex A-50 column (30 cm × 3 cm). Elution was carried out with a gradient increasing concentrations of NaCl solutions (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 1.6, 1.8, 2.0, and 2.5 M) and 3 mL fractions were collected. Elution was monitored by the Dubois method (Chaplin, 1986). Fraction 1 was submitted to total acid hydrolysis as previously described and the constituent monosaccharides were analyzed by GLC as alditol acetates (Chandía and Matsuhiro, 2008).

2.4. Partial hydrolysis of fucoidan from Lessonia vadosa

2.4.1. Partial acid hydrolysis

Native polysaccharide from *L. vadosa* (0.500 g) was stirred with 50 mL of a 0.5 M HCl solution at 60 °C. Aliquots were taken at