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Research paper

# Structure and anticancer activity of native and modified polysaccharides from brown alga *Dictyota dichotoma*

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ABSTRACT

The laminaran DdL and fucoidan DdF were obtained from the brown alga *Dictyota dichotoma*. DdF was a sulfated (28.9%) and acetylated heteropolysaccharide containing fucose, galactose, mannose and glucose (57.9, 20.4, 12.4 and 9.2 mol%, respectively). DdL was a 1,3;1,6-β-D-glucan with the main chain built from 1,3-linked glucose residues and single glucose residue in branches at C6 (one branch on three glucose residues of the main chain). Sulfated (43.7%) laminaran DdLs was obtained from DdL by sulfation. It was determined that sulfates occur at C2, C4 and C6 of glucose residues. The anticancer effect of DdF, DdL, and DdLs (200 μg/mL) was studied *in vitro* on colon cancer cells HCT-116, HT-29, and DLD-1. The effect of polysaccharides (40 μg/mL) on colony formation of DLD-1 cancer cells after irradiation (4 Gy) was investigated first. All polysaccharides showed a synergistic effect with X-ray irradiation against cancer cells, decreasing the amount and size of cancer cells colonies.

## 1. Introduction

Fucoidans are the sulfated algal polysaccharides, which exhibit a wide spectrum of biological activitity, including immunomodulatory, anticoagulant, anticancer, radioprotective and antiviral properties (Ale, Mikkelsen, & Meyer, 2011; Kusaykin et al., 2008). Additionally, fucoidans are non-toxic to organisms and have no side effects. Substances possessing these properties can be used in combined cancer therapy, which is comprised of surgical, radiation and medicinal treatment. There are only a few studies devoted to the radioprotective activity of fucoidans (Byon et al., 2008; Lee et al., 2008; Lee, Bae, Cho, & Rhee, 2009; Qiong et al., 2011), although these polysaccharides are surely prospective non-toxic radioprotectors.

The brown algae *Dictyota dichotoma* belongs to the family Dictyotaceae (order Dictyotales). The algae from this order – *Canistrocarpus cervicornis* (Camara et al., 2011), *Dictyopteris delicatula* (Magalhaes et al., 2011), *D. plagiogramma* (Percival, Rahman, & Weigel, 1981), *D. polypodioides* (Sokolova, Ermakova, Awada, Zvyagintseva, & Kanaan, 2011), *Dictyota dichotoma* (Abdel-Fattah, Hussein, & Fouad, 1978; Hussein, Fouad, & Abdel-Fattah, 1979; Rabanal, Ponce, Navarro, Gomez, & Stortz, 2014), *D. menstrualis* (Albuquerque et al., 2004), *Lobophora variegata* (Medeiros et al., 2008), *Padina gymnospora* (Silva et al., 2005), *P. pavonica* (Hussein, Abdel-Aziz, & Salem, 1980; Men'shova et al., 2012), *P. tetrastromatica* (Karmakar et al., 2009; Rao, Sastry, & Rao, 1984), *Spatoglossum schroederi* (Leite et al., 1998; Rocha et al., 2005), *Stoechospermum marginatum* (Adhikari et al., 2006) – produce predominantly heterogeneous fucoidans. Polysaccharides from *D. dichotoma* have been obtained and investigated by some research groups (Abdel-Fattah, Hussein, & Fouad, 1978; Hussein, Fouad, & Abdel-Fattah, 1979; Rabanal, Ponce, Navarro, Gomez, & Stortz, 2014). It was shown that obtained fucoidans are sulfated heteropolysaccharides. The system of fucoidans (more than 60 fractions), containing fucose, galactose, mannose, xylose, glucose, rhamnose, arabinose and uronic acid residues, was obtained and investigated in study (Rabanal, Ponce, Navarro, Gomez, & Stortz, 2014).

Although fucoidans from this alga have been characterized, there are no data on the isolation and structural characterisation of other algal polysaccharides – laminarans in the literature sources. The laminarans (1,3;1,6-β-D-glucans) are also of interest due to their biological effects and non-toxicity. Earlier, we established that chemical and enzymatic modification of laminarans can improve the biological activity of derivatives in comparison with native polysaccharides (Elyakova et al., 2007; Malyarenko et al., 2017; Menshova et al., 2014; Zvyagintseva, Elyakova, & Isakov, 1995;).

Thus, the aim of this work was to investigate the structural

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characteristics of polysaccharides from *Dictyota dichotoma*, to modify the native laminaran, and to study the anticancer and radioprotective activities of fucoidan, laminaran and its sulfated derivative.

## 2. Experimental

### 2.1. Materials

Organic solvents, inorganic acids and salts, sodium hydroxide and trifluoroacetic acid (TFA) were commercial products (Laverna-Lab, Moscow, Russia). Standards (mannose, rhamnose, glucose, galactose, xylose and dextrans) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Sorbents for chromatography were Polychrome-1 (Reakhim, Moscow, Russia), DEAE-cellulose Sigma–Aldrich (St. Louis, MO, USA), Macro-Prep DEAE (Bio-Rad Laboratories, Hercules, CA, USA) and Amberlite CG-120 (Serva Electrophoresis GmbH, Heidelberg, Germany).

Basal Medium Eagle (BME), McCoy's 5A Modified Medium (McCoy's 5A), RPMI-1640 Medium, trypsin, fetal bovine serum (FBS) and agar were purchased from Gibco/Life Technologies (Carlsbad, CA, USA). MTS reagent – 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide was purchased from Promega Corporation (Madison, WI, USA). Phosphate buffered saline (PBS), L-glutamine and penicillin–-streptomycin solution (10000 U/mL, 10 μg/mL) were from Sigma–Aldrich (St. Louis, MO, USA).

Human colorectal adenocarcinoma HCT-116 (ATCC® no. CCL-247™), HT-29 (ATCC® no. HTB-38™), DLD-1 (ATCC® no. CCL-221™) cell lines were obtained from the American Type Culture Collection (Manassas, VA, USA).

A sample of the algae *Dictyota dichotoma* (Dd) was collected from Peter the Great Bay in August 2014, Sea of Japan (Russia). Fresh algal biomass (100 g) was powdered and pre-treated with 70% aqueous ethanol (w/v = 1:10) at room temperature for 10 days. Defatted alga was air-dried.

### 2.2. Instruments

NMR spectra were obtained on an Avance DPX-500 NMR spectrometer (Bruker BioSpin Corporation, Billerica, MA, USA) resonating at 75.5 MHz at 35 and 60 °C. The sample concentration was 15 mg of polysaccharide/mL of $D_2O$ for 1D and 2D experiments.

### 2.3. General methods

#### 2.3.1. Analytical procedures

Total carbohydrates were quantified by the phenol-sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Monosaccharide composition was determined by HPLC with an ISA-07/S2504 column (0.4 × 25 cm, Shimadzu, Kyoto, Japan), a bicinchoninate assay and a C-R2 AX integrating system (Shimadzu, Kyoto, Japan) after hydrolysis with 2 M trifluoroacetic acid (6 h, 100 °C). Monosaccharides (rhamnose, ribose, mannose, fucose, galactose, xylose, and glucose) were used as standards for HPLC. The protein and polyphenol contents were determined using the Bradford method (Bradford, 1976) and a modification of the Folin–Ciocalteau method (Singleton & Rossi, 1965), respectively. The amount of sulfate groups was determined by using the $BaCl_2$ gelatin method (Dodgson, 1961).

#### 2.3.2. Molecular weight determination

Samples of polysaccharides were analyzed using a high-performance liquid chromatography instrument (Agilent 1100 Series, Germany) equipped with a refractive index detector and a series-connected gel-filtration column (TSK gel G4000 SW and TSK gel G2000 SW, Tosoh Co., Tokyo, Japan). Elution was performed with a 0.05 M aqueous solution of $Na_2SO_4$ at 50 °C with a flow rate of 0.5 mL/min. The molecular weights of polysaccharides were estimated using dextrans of molecular weights 5, 6, 10, 40, 70, 100, 200 and 450–650 kDa as reference standards.

#### 2.3.3. Polysaccharide extraction

Samples of defatted, dried and powdered algal fronds (100 g) were extracted twice with 0.1 M HCl (2 L) for 2 h at 60 °C. The extracts were combined, centrifuged, dialyzed, concentrated on a rotary evaporator (2.5 h, 45 °C) and lyophilized to obtain the polysaccharide fraction DdP (8.4 g).

#### 2.3.4. Anion-exchange chromatography of polysaccharides on DEAE-cellulose

A solution of polysaccharide DdP in 50 mL of 0.1 M HCl was applied to a DEAE-cellulose column ($Cl^-$ form, 21 × 3 cm) equilibrated with 0.1 M HCl. The laminaran-containing fraction was eluted with water, neutralized and concentrated on rotary evaporator (40 min, 45 °C). Then, the column was successively washed with 1 and 2 M NaCl solutions until the disappearance in the eluate of a positive reaction for carbohydrates by the phenol-sulfuric acid method (Dubois et al., 1956) in each case. The fraction containing fucoidan was eluted with 2 M NaCl, then concentrated on rotary an evaporator (40 min, 45 °C), dialyzed and lyophilized with a yield of 1.1 g.

#### 2.3.5. Hydrophobic chromatography of laminaran on Polychrome-1

The solution of laminaran was applied to a Polychrome-1 column (15 × 4.5 cm). The column was subsequently washed with water and 5% aqueous ethanol. Then, the pure laminaran (L) DdL was eluted with 15% aqueous ethanol until the disappearance in the eluent of a positive reaction for carbohydrates by the phenol-sulfuric acid method (Dubois et al., 1956). The eluate was concentrated on a rotary evaporator (40 min, 45 °C) and lyophilized. The yield of the laminaran fraction DdL was 1.3 g.

#### 2.3.6. Removal of polyphenols from the fucoidan fraction

A sample of the fucoidan fraction was dissolved in water (100 mL), to which was added 30% aqueous $H_2O_2$ (20 mL), and then 10% aqueous $NH_3$ until to the solution reached pH 8.5. The resulting mixture was kept at room temperature for 17 h in the dark. Then, the solution was centrifuged, and the supernatant was dialyzed, concentrated on a rotary evaporator (40 min, 45 °C) and lyophilized to obtain the pure fucoidan fraction DdF with a yield of 739 mg.

#### 2.3.7. Anion-exchange chromatography of fucoidan on Macro-Prep DEAE

A solution of fucoidan in 0.1 M NaCl (0.5 g in 10 mL) was applied to a Macro-Prep DEAE (Bio-Rad, USA) column ($Cl^-$ form, 2.5 × 9 cm) equilibrated with 0.1 M NaCl. Then the column was successively eluted with a linear gradient of NaCl (from 0.1 to 2 M). The eluate was analyzed by the phenol-sulfuric acid method (Dubois et al., 1956). The obtained fraction of fucoidan DdF was concentrated on a rotary evaporator (40 min, 45 °C), dialyzed and lyophilized with a yield of 415 mg.

#### 2.3.8. Sulfation of laminaran

Pyridine (30 mL) in a bottle was cooled in an ice bath, and chlorosulfonic acid (10 mL) was slowly dropped into the pyridine solution and incubated for 1 h to form a mixture. The laminaran DdL (1 g) was dissolved in 100 mL of dimethylformamide, and the resulting solution was added to the mixture. Then, the bottle with reagents was kept in a hot water bath at 75 °C for 1.5 h. Subsequently, the solution was cooled, resolved in water (250 mL) and neutralized with NaOH. The obtained sulfated (s) laminaran DdLs was precipitated by 96% ethanol and centrifuged. The precipitate was dissolved in water, dialyzed and lyophilized with a yield of 377 mg.