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Gelling polysaccharide from *Chondrus armatus* and its oligosaccharides: The structural peculiarities and anti-inflammatory activity

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ABSTRACT

Structural peculiarities of low molecular weight (LMW) sample obtained by mild acid hydrolysis of κ-carrageenan from *Chondrus armatus* was investigated by a rapid mass spectrometric method. The selected conditions allowed avoiding excess destruction of 3,6-AnGal residues that was shown by using tandem (MS/MS) mode. Main oligosaccharide fraction with molecular weight 2.3 kg/mol, obtained by mild acid hydrolysis was chosen for the analysis. It was shown that fragments with even degree of polymerization (DP) were mostly built of $(\text{-G4S-DA-})_n$ repeating unit, $n = 1–5$. Some fragments with odd DP were shown to contain -DA2S- insertions, being the fragments of ι-type blocks, which were randomly distributed along the polysaccharide chain as single insertions. The anti-inflammatory activity (on *acetic acid-induced* colitis in mice) of the initial polymer and its derivatives was studied. The anti-inflammatory effect of the polymer was observed at a dose of 5 mg/kg. Polysaccharide decreased the degree of colon damage more than twice and area of damage in 40%.

1. Introduction

Red algae are the source of interest on the structure, physico-chemical properties and biological activity of polysaccharides–carrageenans. Carrageenans are a family of natural water-soluble linear sulfated galactans extracted from numerous species of red algae. They are composed of alternating 3-linked β-D-galactopyranose and 4-linked α-D-galactopyranose or 4-linked 3,6-anhydrogalactose forming the disaccharide repeating unit of carrageenans classified according to the number and the position of sulfated esters (S) and by the occurrence of 3,6 anhydro-bridges in the α-linked residues (DA unit) found in gelling galactans (Knutsen, Myslabodski, Larsen, & Usov, 1994). For example, the three most industrially exploited carrageenans, namely κ—(DA-G4S), ι—(DA2S-G4S) and λ—(D2S6S-G2S) carrageenans are distinguished by the presence of one, two and three ester-sulfate groups per repeating disaccharide unit, respectively. Native carrageenans always represent complex hybrid structures or are generally a mixture of galactans composed of different carrabiose types, the proportions and structures of which vary with species, ecophysiological and development conditions (Falshaw & Furneaux, 1994; Knutsen et al., 1994; Yermak & Khotimchenko, 2003).

It is known that carrageenans have a wide application in practice. In food industry, carrageenans are widely utilized due to their excellent physical functional properties, such as thickening, gelling and stabilizing abilities. The safety of carrageenans for use in foods was confirmed at the 57th meeting of the JECFA (2008)—Joint Food and Agriculture Organization of the United Nations/World Health Organization Expert Committee in Food Additives. According to the JECFA, only degraded carrageenans were associated to adverse effects and should not be used as food additives.

Recently, the carrageenans are of importance in pharmaceutical development. Carrageenans were used to reduce the amount of polymorphic transformation in tableting (Schmidt, Wartewig, & Picker, 2003), to produce controlled release delivery system (Thommes & Kleinebudde, 2006), and to achieve interactions with drugs for modified release systems (Hugerth, 2001).

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Intensive studies showed that carrageenans can be regarded not only as foodstuff ingredients, but also as the drugs due to a wide spectrum of biological and physiological activities, such as antiviral (Baba, Snoeck, Pauwels, & Declercq, 1988; Ghosh et al., 2009), anticoagulant (Yermak et al., 2012; Zuniga, Matsuhiro, & Mejias, 2006), antitumor (Yuan, Song, Li, Li, & Liu, 2011), and immunomodulatory (Bhattacharyya et al., 2010; Yermak et al., 2012). Carrageenans also possess immunoadjuvant properties, and they can exert both immunopotentiative and immunosuppressive actions (Bhattacharyya et al., 2010).

There are many evidences indicating that the number of sulfate groups and molecular weight of seaweed polysaccharides were important factors in influencing their biological action Yermak & Khotimchenko, 2003. Recently, it was reported that the low molecular weight fractions of carrageenan from *Solieria chordalis* possess great immunostimulating properties (Bondu, Deslandes, Fabre, Berthou, & Yu, 2010). In addition, usually, the antiviral activity of polysaccharides correlates with their molecular weight. The higher average molecular weight, the higher antiviral activity for many cases (Ghosh et al., 2009). In our investigation of antiviral activity of different structural types of carrageenans and their low molecular weight derivatives obtained by different hydrolysis methods it was shown that polysaccharides posses the higher effect against tobacco mosaic virus was in comparison with their oligosaccharides independently on the depolymerization way (Kalitnik et al., 2013). Moreover, pharmacological activity of carrageenans is dose-dependent and depends on the structural type of polysaccharide (Craigie, 1990; Yermak & Khotimchenko, 2003). Early we showed that the ability of carrageenans to influence on the cytokine production by human cells is greatly dependent on structure of polysaccharides (Yermak et al., 2012). Moreover, the relationship between chemical structure, molecular weight, also dose of carrageenans and their biological activity was still not being studied fully. Hence, various modification processes including depolymerisation is important to determine the structural unites, which are primary ones for biological activities of polysaccharides and it have an important value to prepare the drugs with necessary properties.

The purpose of the current study was to investigate the structural peculiarities of low-molecular-weight (LMW) derivative obtained by mild acid hydrolysis of κ-carrageenan from *Chondrus armatus* and their anti-inflammatory activity (on *acetic acid-induced* colitis in mice) in comparison with the initial polymer.

2. Experimental

2.1. Materials

Red alga *C. armatus* (Gigartinaceae) was collected at the Peter the Great Bay, Sea of Japan. The κ-carrageenan was extracted and purified as described earlier (Barabanova et al., 2005; Yermak, Kim, Titlynov, Isakov, & Solov'eva, 1999).

All MS experiments were performed using ultra pure water, produced with Millipore Direct-Q 3 equipment (USA). A MALDI matrix (2,5-dihydroxybenzoic acid, DHB) was purchased from Sigma (USA).

2.2. Instruments

Monosaccharides as alditol acetate derivatives were identified by gas chromatography (GLC) using an Agilent 6850 gas chromatograph (USA) equipped with a HP-5MS capillary column (30 m × 0.25 nm) with 5% Phenyl Methyl Siloxan and flame ionization detector. The analyses were carried out at temperature done as suggested programming from 175 to 225 °C with 3°/min.

IR spectrum of the polymer sample was recorded using a Vector 22 Fourier transform spectrophotometer (Bruker, Germany) with resolution of 4 cm^{-1}. The spectra of polysaccharide and their LMW derivatives were recorded in KBr pellets. The spectra were normalized by the absorption of the monosaccharide ring at ~1070 cm^{-1} relatively local base line in region at ~1500–900 cm^{-1}.

MALDI-TOFMS spectra were recorded with UltraFlex III MALDI-TOF/TOF mass spectrometer (Bruker, Germany) with nitrogen laser (337 nm), reflector and LIFT modes of operation. Instrument settings for the negative-ion mode: accelerating voltage, −25 kV; lase power, 19%; number of shots, 200; laser shot rate, 66 Hz. Sample preparation: 2 μL of the mixture, containing 0.5 μL of the sample (1 mg/mL) and 0.5 μL of L-Fuc (10 mg/mL) solutions in water, 1 μL of DHB matrix (50 mg/mL) in 1:1 acetonitrile–water solution was mixed and introduced onto the sample plate and air dried.

2.3. General methods

The total amount of carbohydrates was estimated by the phenol-sulfuric acid method, using D-galactose as a standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956).

The content of 3,6-anhydrogalactose was determined according to the method of Usov and Elashvili (1991). Polysaccharide (5 mg) was placed in a test tube with borane-4-methyl-morpholine (40 mg) (Sigma, USA) and aqueous trifluoracetic acid (TFA) (2 M, 1 mL) containing mio-inositol (Acros Orcanics, USA) as internal standard (1 mg/mL). The solution was heated at 100 °C for 4 h. The solvent was evaporated and three portions of 3 mL of 96% ethanol were added to the hydrolysate and evaporated to remove traces of TFA. A hydroxylamine hydrochloride (6%, 1 mL) was added to the vacuum-dried hydrolysate residue. The solution was heated at 100 °C for 1 h. After cooling, acetic anhydrate (1 mL) was added and the tube was heated at 100 °C for 1 h. Toluene (3 mL) was added three times and the solvent was evaporated to dryness. Then CH_3Cl (5 mL) and water (5 mL) were added. After shaking, the chloroform layer was separated and evaporated to almost dryness.

The content of sulfates was determined by a turbidimetric method of Dodgson and Price. The method is based on precipitation of inorganic sulfate by barium chloride–gelatin reagent in the presence of 4% trichloroacetic acid (Dodgson & Price, 1962).

Analysis of monosaccharide composition of polysaccharide and oligosaccharide fractions was performed by alditol acetate method (Sawardeker, Sloneker, & Jeanes, 1965). The total acid hydrolyses were carried out with 2 M trifluoroacetic acid at 105 °C for 4 h followed by reduction in H_2O with $NaBH_4$ overnight at room temperature.

2.3.1. Degradation of κ-carrageenan by mild acid hydrolysis

Mild acid hydrolysis was conducted by the method of Yu et al. (2002). Carrageenan (200 mg) was dissolved in 0.1 M hydrochloric acid (20 mL) and kept for 24 h at 37 °C. The depolymerization reaction was terminated by neutralization with 0.1 M NaOH. Then oligosaccharides were precipitated with 5 volumes of ethanol and centrifuged at 10.000 rpm for 20 min at 4 °C. The pellet was collected and lyophilized.

2.3.2. Gel-permeation chromatography

Oligosaccharides (pellet) were purified by gel filtration on a Biogel P-6 column (24.5 × 1.5 cm). The volume of samples injected was approximately 2 mL of H_2O (15 mg). The column was eluted with distilled H_2O using the pump with a flow rate of 0.8 mL min^{-1}. Fractions were collected with a BioRad fraction collector (USA) per

2.5 mL. The fraction with highest yield (fraction 2, **LMW-carr.**) was detected by the phenol-sulfuric method (Dubois et al., 1956).

2.3.3. Molecular weight estimation

Viscosimetric molecular weight of carrageenan sample were calculated using the Mark–Houwink equation: $[\eta] = KM^{\alpha}$, where $[\eta]$ is the intrinsic viscosity and K and α are empirical constants for κ-carrageenans constituting 3×10^{-3} and 0.95 at 25 °C in 0.1 M NaCl, respectively, according to the literature data for this polymer–solvent system (Rochas, Rinaudo, & Landry, 1990). The viscosity of carrageenan solutions (0.1–1.0 mg mL^{-1} in 0.15 M NaCl) was measured in a modified Ubellode viscometer with a capillary diameter of 0.3 mm at 26 °C, the time of accuracy being within ±0.1 s. The intrinsic viscosity of the carrageenan samples was calculated by the etrapolation of the dependence ln(η)rel/C to infinite dilution using the least square method.

The molecular weight of **LMW-carr.** sample derivative was determined by the reducing sugars method with ferricyanide (Park & Johnson, 1949).

2.4. Anti-inflammatory activity

2.4.1. Induction of experimental colitis

Male Swiss mice weighing 20–25 g were used. They were housed under standard environmental conditions and fed with rodent diet and water ad libitum. Mice were deprived of food a night before induction of colitis. Colitis was induced by intrarectal administration of 100 μL 5% acetic acid (pH 2.5) 3 cm from the anal margin (Itoh et al., 2000). Colitis was then induced and its severity was evaluated morphologically and biochemical (myeloperoxidase) parameters. Each experimental group consisted of seven animals.

2.4.2. Application of carrageenans

Animals were treated orally with carrageenans at the dose of 5, 10, 50 mg/kg dissolved in water using a flexible catheter 24 h before induction of colitis. Control mice received the same amount (0.2 mL) of vehicle. Positive control and reference groups received prednisolone (5 mg/kg)

2.4.3. Assessment of colitis severity

Mice were killed by cervical dislocation 1 day after administration of acetic acid. The entire colons were isolated, opened longitudinally, and rinsed with phosphate-buffered saline (PBS). Macroscopic scoring of the colon damage was performed using the following criteria: 0, no macroscopic change; 1, mucosal erythema alone; 2, mild mucosal edema, slight bleeding, or small erosion; 3, moderate edema, bleeding ulcers or erosion, edema, and tissue necrosis (Mahgoub, El-Medany, Hager, Mustafa, & El-Sabah, 2003). For each mouse, the ulcer area was determined by summing the sizes of lesions measured macroscopically by two blinded observes. The total area of damage was expressed as the relative percentage of the total surface area of the colon.

2.4.4. Determination of colonic myeloperoxidase (MPO) activity

After the macroscopic measurements, the excised colons (100–150 mg) were homogenized in PBS (pH 7.4) and centrifuged at 10 000 *g* for 20 min at 4 °C. MPO activity in supernatants was then assayed by mixing the supernatant with citric phosphate buffer (pH 5.0) containing 0.4 mg/mL *o*-phenylene diamine and 0.015% hydrogen peroxide. The change in absorbance (OD) at 492 nm was measured spectrophotometrically and compared with a standard dilution with horseradish peroxidase (Evans, Laszlo, & Whittle, 2000). MPO activity is expressed as units per milligram of tissue.

2.5. Data analysis

Results are given as mean ± SD. The significance of differences between means was evaluated by Student's *t*-test; *n* = number of animals.

3. Results and discussion

3.1. Preparation and characterization of oligosaccharides

The polysaccharide–κ-carrageenan from *C. armatus* (average MW 250 kg/mol, Section 2.3.3) was used to obtain LMW fragments. The chemical structure of the polysaccharide was determined earlier (Yermak et al., 1999).

Mild acid hydrolysis (Section 2.3.2) products were fractionated by gel-permeation chromatography (Section 2.3.1). **LMW-carr.** oligosaccharide fraction with MW 2,3 kg/mol (Table 1) was used for the analysis. The monosaccharide composition, sulfate content and molecular weight of **LMW-carr.** as well as MW of the polymer are listed in Table 1.

According to the data of IR-spectroscopy and chemical analysis the structural features of carrageenan oligosaccharides were determined to correspond with the initial polysaccharide. The IR spectra of the **LMW-carr.** sample are contained characteristic absorption bands for κ-carrageenan (Stancioff & Stanley, 1969). It was calculated the ratios of absorbance value, characteristic for sulfate, 3,6-AnGal content and monosaccharide rings.

As shown in Table 1, a mild acid hydrolysis of carrageenan results to a decrease of 3,6-AnGal amounts in comparison with the initial polymer. This fact could be explained by the partial destruction of 3,6-AnGal by acid action (Usov & Elashvili, 1991). The sulfate content of oligosaccharides was also lower due to the partial loss of labile sulfate groups during acid hydrolysis. The analysis of the IR spectra supports the data of chemical results (Table 1).

3.2. Mass spectrometry of *LMW-carr.* oligosaccharide fraction

Yang et al. studied the mechanism of mild acid hydrolysis of κ-carrageenan in details (Yang et al., 2009). It was shown that acid hydrolysis at 60 °C results to a cleavage of α-1,3-linkages and production of the odd-numbered oligosaccharide fragments, while even-numbered fragments were produced after breaking of

Table 1
The monosaccharide composition and sulfate content of κ-carrageenan and oligosaccharide fraction.

Carrageenan	Hydrolysis reagent	Content					MW (kg/mol)
		Chemical analysis (% dry weight)			IR spectroscopy		
		Gal	3,6-AnGal	SO_4^{2-}	3,6-AnGal**	SO_4^{2-}*	
HMW-carr. (*C. armatus*)	–	30	27	22	0.38	0.59	250
LMW-carr.	HCl 0.1 N 24 h 37 °C	27	22.5	21	0.18	0.58	2.3

MW—molecular weight.

* A_{1258}/A_{1070}.

** A_{930}/A_{1070}.

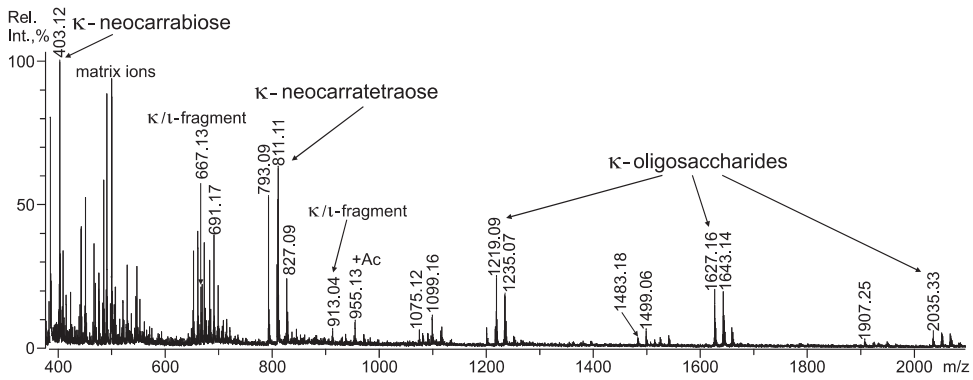

Fig. 1. Negative-ion MALDI-TOFMS of the LMW mixture of oligosaccharides obtained by acid hydrolysis of polysaccharide from *C. armatus.*

β-1,4-linkages and were detected as minor components. In our experiment acid hydrolysis was carried out at 37 °C and thus we obtained conditions that allowed us to avoid excess destruction of 3,6-AnGal residues resulting in raise of yields of oligosaccharides with even degree of polymerization.

A negative-ion mass spectrometry (MALDI-TOFMS) was applied to investigate the composition and structural features of oligosaccharide mixture obtained from κ-carrageenan of *C. armatus* by mild acid hydrolysis. It was found (Fig. 1) that oligosaccharide mixture contained intensive molecular ions of "ideal" oligosaccharides, built up of κ-blocks, where the number of monosaccharides was even. Mass spectrum also contained less-intensive signals from the losses in the ion source, the loss of water molecules (like *m/z* 793.09 and similar, like "811.11 – 18 g/mol") and the loss of $NaHSO_4$ (*m/z* 691.17, "811.11 – 120 g/mol"). In addition, mass spectrum (Fig. 1) contained signals of the ions, corresponding to single ι-insertions into κ-carrageenan (*m/z* 667.13 and a minor 913.04). The fact was observed in both acid hydrolysis products by ESIMS/MS (Yu et al., 2006) and κ-carrageenase action products (Anastyuk et al., 2011). "Plus 16" ion signals (1235.07 and others) are assumed to arise from an exchange from sodium to potassium in sulfate salts of oligosaccharides. It must be noted that oligosaccharide mixture also contained a small signal at *m/z* 259.01 of monosulfated galactose residue.

The application of potential LIFT MS/MS mode of operation allowed us to identify the structural particularities of some oligosaccharides directly from the mixture (Table 2). The fragmentation pattern of the κ-carrageenan-derived oligosaccharides, released by mild acid hydrolysis and by enzymatic treatment was recently studied (Yu et al., 2006). It was shown that collisionally-induced dissociation negative-ion electrospray ionization tandem mass spectrometry (CID ESIMS/MS) of "sodiated" (Na^+ form) oligosaccharides featured extensive series of B- and C-type glycosidic cleavages, while the Y-type cleavage occurred mainly at the sulfated residues (according to the nomenclature, suggested by Domon and Costello (1988). The assignment of reducing or non-reducing terminal fragments was assisted by oligosaccharide reduction and the product ion spectra of the derived alditols. In our case a negative-ion tandem MALDI-TOFMS has an advantage over ESIMS technique mentioned above as the complex sample preparation/introduction procedure is omitted, since there is no need in extra "sodiation" of the sample. The MALDI matrix does that automatically, as well as the production of the singly-charged ions. The decomposition pattern practically remains the same. The only difference that B_2- and C_2-type ion signals in our case are barely visible (Fig. 2B). The fragmentation pattern (Fig. 2A) of the tetrasaccharide at *m/z* 811.11 from the sample under study exhibits similar fragmentation patter as was observed in the work of Yu et al. (2006), suggesting the tetrasaccharide to have G4S-O-DA-O-G4S-O-DA structure. However, author did not establish the nature of the ion at *m/z* 505, which was also detected in our sample in the odd-numbered ion at *m/z* 667.13 (see below).

The odd-numbered fragments which were observed in the mixture contained Gal-4-OSO_3 residue (Fig. 3) at the non-reducing terminus (tandem mass spectrum of the ion at *m/z* 1075.12 is available as a supplementary material). The oligosaccharides with even DP (degree of polymerization) contained 3,6-AnGal (data not shown) residues at the non-reducing terminus. The tandem mass spectra were similar to that observed earlier by Yu et al. (2006). That is in agreement with literature data (Bondu et al., 2010; Myslabodski, Stancioff, & Heckert, 1996). The fragment at *m/z* 667.13 also had a variant, suggesting a minor "impurity" of ι-type (Fig. 3) because of the presence of a Y'_2-type fragment ion at *m/z* 505.0. We believe that *m/z* 505.0 in the case of both precursor ions at *m/z* 811.11 and 667.13 comes from (G-O-DA-G4S-DA2S) insertion. Unsulfated galactose residue on the non-reducing end, probably, comes from either a loss of a sulfate in the ion source or during hydrolysis. An ι-containing variant of the ion at *m/z* 617.13, probably, came from G-O-DA-G4S-DA2S fragment at *m/z* 811.11, where AnGal residue was destroyed under acidic conditions.

Thus, mass spectrometry was successfully used to characterize oligosaccharide mixture, obtained by acid hydrolysis in mild conditions from κ-carrageenan. Fragments with even DP (most abundant due to mass spectral data) were shown to be mostly built of (-G4S-DA-)$_n$ repeating unit, $n = 1–5$. Some fragments with odd DP were shown to contain -DA2S- insertions, being the fragments of ι-type blocks, which were randomly distributed along the polysaccharide chain as single insertions.

Table 2
The structural features of some κ-carrageenan oligosaccharides obtained by mild acid hydrolysis elucidated by negative-ion MALDI-TOFMS/MS (tandem mode of operation).

m/z	Composition and structure of oligosaccharides
259.01	G4S
403.12	G4S-DA, DA-G4S
667.13	G4S-DA-G4S, G-DA2S-G4S
811.11	G4S-DA-G4S-DA
1075.11	DA-G4S-DA-G4S-DA
1219.09	G4S-DA-G4S-DA-G4S-DA

3.3. *The effect of κ-carrageenan and* ***LMW-carr.*** *fraction on acetic acid-induced colitis*

It is known that sulfated polysaccharides of marine algae decrease leucocyte migration to locus of inflammatory, exhibit anti-complementary activity (Davies, 1965) and suppression activity of connective tissue proteolytic enzymes such as heparanase and elastase (Parish, Freeman, & Hulett, 2001; Senni et al., 2006).

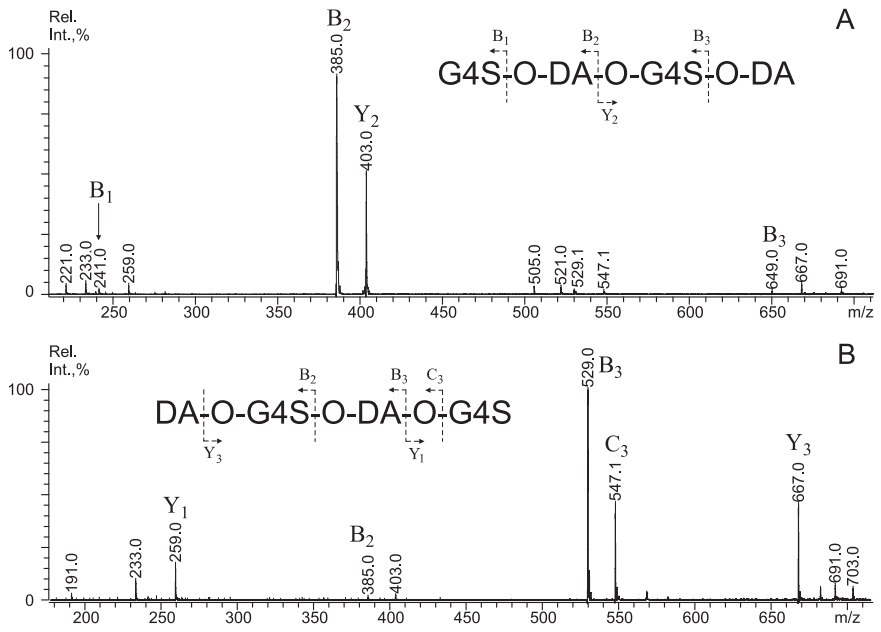

Fig. 2. Negative-ion tandem MALDI-TOFMS of ion at *m/z* 811.11 (A) obtained by mild acid hydrolysis from κ-carrageenan under study, (B) obtained by enzymatic digestion (recombinant *Alteromonas carrageenovora* κ-carrageenase) of κ-carrageenan standard purchased from Sigma (USA).

It may be useful to prevent human organism from different pro-inflammatory statuses.

Plant polysaccharides have been previously demonstrated to reduce colon damage in experimental colitis (Lim, Lee, Park, & Choue, 2003; Liu et al., 2003; Rolandelli et al., 1988) So, pectic polysaccharides are well known to be a component of dietary fiber and to possess immunomodulating capacity with a predominant effect on neutrophils and macrophages. Feeding with pectins has been found to reduce a degree of experimental bowel injury induced by acetic acid (Rolandelli et al., 1988) Popov S. V. et al. shown that oral pretreatment with comaruman, a pectin from cinquefoil *Comarum palustre* L., has been shown to prevent the development of experimental colitis in mice (Popov, Ovodova, Markov, Nikitina, & Ovodov, 2006).

Anti-inflammatory effect of κ-carrageenan (high molecular weight—**HWM-carr.**) and oligosaccharides obtained by mild acid hydrolysis (**LWM-carr.**) was assessed using the following criteria: the degree of damage of wall colon and the total area of its damage and colonic myeloperoxidase (MPO) activity. The doses of carrageenan used were chosen to approximate the estimated human intake as dietary fiber (Englyst, Quigley, & Hudson, 1995). The extent of the preventive effect of polysaccharide was comparable with that of prednisolone, a hormonal anti-inflammatory drug. It was shown **HWM-carr.** had a protective effect on the progression of colitis in mice at a dose of 5 mg/kg under oral administration. As our results, shown polysaccharide decreased the degree of colon damage more than twice and area of damage in 40% (Fig. 4A and B).

In addition, **HWM-carr.** sample at a dose of 5 mg/kg suppressed the tissue MPO activity in colons in 2.9 times (Fig. 5).

The effect of carrageenan on colon inflammation in mice was dose-related. A pronounced anti-inflammatory effect of **HMW-carr.** sample was observed at a dose of 5 mg/kg, whereas at a dose of 10 mg/kg polysaccharide does not possessed a protective effect, and increase in the dose up to 50 mg/kg leaded to increase inflammation of the colon in mice (Fig. 4). These results are agreement with a number of studies indicating that large amounts of oral carrageenan induce colitis (Anver & Cohen, 1976; Watt & Marcus, 1973), while small quantities do not appear to be toxic for the gut mucosa (Nicklin & Miller, 1984).

According to our results, a LMW-product of acid hydrolysis of κ-carrageenan did not show any protective effect at a dose of 5 mg/kg, whereas a dose of 50 mg/kg, as well as the native polysaccharide, stimulated the development of inflammation (Fig. 4).

It known that degraded low molecular weight carrageenan exhibits toxicological properties at high doses, stimulates ulceration of the cecal mucosa, histopathological changes, epithelial thinning, slight erosion, cellular infiltration and other negative changes in animal organisms (Cohen & Ito, 2002). It is possible that

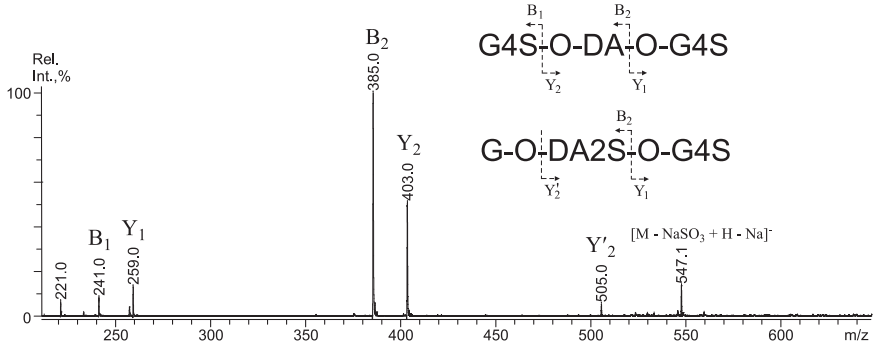

Fig. 3. Negative-ion tandem MALDI-TOFMS of ion at *m/z* 667.13.

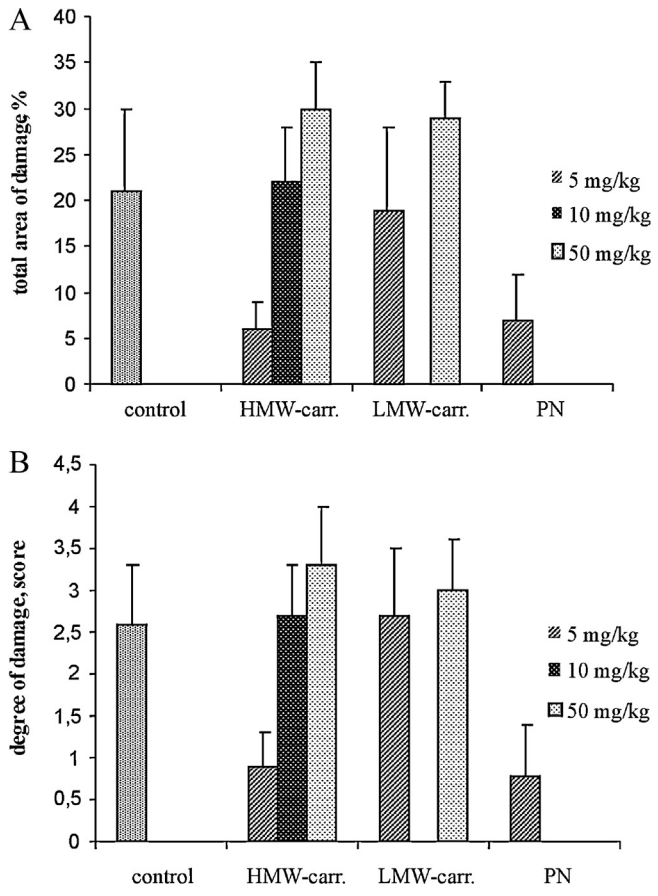

Fig. 4. The total area of damage of wall colon (A) and degree of its damage (B) under oral administration carrageenan 24 h before induction of colitis. Values are means ± SD ($n = 7$), $P < 0.05$. Control—0.9% NaCl, PN—prednisolone.

carrageenan oligosaccharides (**LMW-carr.**) do not possess protective effect on the progression of colitis in mice, and stimulated the development of inflammation due to their toxicological properties and ability to cause of ulcerative colitis or erosions of the mucous membrane of caecum and colon in animal organisms.

At the same time, according to data of Joint FAO/WHO and Expert Committee on Food Additives (JECFA), native high molecular weight carrageenan is safe and non-toxic product (JECFA, 2008). The long safe history of this natural food additive–carrageenan is confirmed by negative results in subchronic and chronic feeding studies, mutagenicity and reproductive toxicity studies in many animal species, not effects body weight, organ weights, urine and fecal characteristics or hematological and blood chemical parameters, no any changes in macroscopic or microscopic appearance of the gastrointestinal tract (Weiner, 1991).

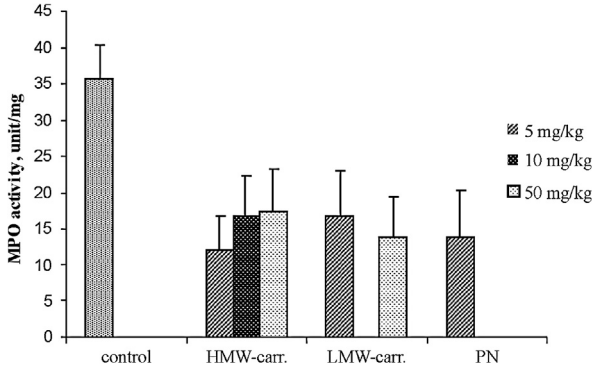

Fig. 5. The myeloperoxidase (MPO) activity in colonic tissue of mice under oral administration of carrageenan 24 h before induction of colitis. Values are means ± SD ($n = 7$). $P < 0.05$.

It is worth noting that as a further precautionary measure European Committee limited the "Acceptable Daily Intake" of carrageenan to 75 mg/kg body weight (Bixler, 1994). Although according to JECFA the average daily intake dose of carrageenan for adults does not exceed 10 mg/kg (JECFA, 2008). In our experiment, the used dose of carrageenan at which increasing inflammation of the colon in mice was observed exceeded the average daily intake safe dose of polysaccharide. This fact could be explained that carrageenan at high dose possess pronounced immunostimulating action. At the same time, immunosuppressive effect of oral carrageenan exhibited at a low dose (5 mg/kg) that is in agreement with literature data about dose-depended immunomodulatory activity of carrageenan (JECFA, 2008).

The exact mechanism of the preventive effect of carrageenan on colitis remains unclear. The suppression of MPO activity by carrageenan may be due to inhibitions the accumulation of neutrophils in the colonic mucosa under action of polysaccharide. Indeed, MPO activity has been previously shown to be proportional to a number of infiltrating granulocytes, primarily neutrophils in the model of colonic inflammation (Krawisz, Sharon, & Stenson, 1984). Increasing amounts of transmigrating neutrophils induce significant epithelial disruptions resulting in epithelial discontinuities and erosions (Nusrat, Parkos, Liang, Carnes, & Madara, 1997) Therefore, we suggest that a delay in the influx of neutrophils into the intestinal wall is implicated in the preventive effect of carrageenan.

The role of reactive oxygen species has been stressed in recent years. Increased lipid peroxidation has been identified in the colons of patients with ulcerative colitis (Koch et al., 2000) as well as in experimental colitis induced by acetic acid (Cetinkaya et al., 2006). Plant polysaccharides, including seaweeds polysaccharides, are assumed capable of minimizing free radical-induced damage (Choi et al., 2010). It was shown that the protective effects of brown alga *Padina boergesenii* extract against carbon tetrachloride induced oxidative damage and liver fibrosis in rats is produced its antioxidant sparing actions (Karthikeyan, Somasundaram, Manivasagam, Balasubramanian, & Anantharaman, 2010).

What is more earlier we shown that the different structural types of carrageenan from red algae of Gigartinaceae and Tichocarpaceae families possesses *in vitro* scavenging effect on hydroxyl radicals, superoxide anion, nitric oxide and hydrogen peroxide (Sokolova et al., 2011), it was proposed that the protective effect of **HMW-**carrageenan against colitis was possibly related in some degree to its antioxidant action.

4. Conclusions

The structural peculiarities of carrageenan, sulfated polysaccharide from red seaweed *C. armatus* were investigated by a rapid mass spectrometric method. The investigation of oligosaccharide mixture, obtained from κ-carrageenan by acid hydrolysis in mild conditions (37 °C) has shown an increase in the yields of fragments with even DP in comparison with literature data, where higher temperature (60 °C) was used (Yang et al., 2009). The selected conditions allowed us to avoid excess destruction of 3,6-AnGal residues, that was shown by using LIFT TOF/TOF mode (MS/MS). The oligosaccharides were found to be mostly built of (-G4S-DA-)$_n$ repeating unit, $n = 1–5$. Some other interesting fragments were also detected in the mixture: fragments with odd DP were shown to contain -G4S-DA2S- insertions, being the fragments of ι-type blocks, which were randomly distributed along the polysaccharide chain as single insertions. Some fragments contained unsulfated galactose residue at the non-reducing end. Probably, a loss of a

sulfate occurred in the ion source of mass spectrometer or during hydrolysis.

We have found that carrageenan from *C. armatus* had a protective effect on the progression of colitis in mice at a dose of 5 mg/kg under oral administration. Polysaccharide decreased the degree of colon damage more than twice and area of damage in 40%. The effect of carrageenan on colon inflammation in mice was dose-related. A pronounced anti-inflammatory effect of polysaccharide sample was observed at a dose of 5 mg/kg, whereas at a dose of 10 mg/kg polysaccharide does not possessed a protective effect, and increase in the dose up to 50 mg/kg leaded to increase inflammation of the colon in mice. Leukocyte adhesion represents one of the first steps in initiation of the inflammatory response and it is essential for the accumulation of active immune cells at sites of inflammation (Wagner & Roth, 1999). The ability of carrageenan to influence the adhesion of peritoneal leukocytes reduction may partially explain their preventive effect on the accumulation of neutrophils in the intestinal wall. Thus, neutrophil infiltration reduction and antioxidant action may be implicated in the protective effect of carrageenan on acetic acid-induced colitis in mice.

The dose-dependent effect of carrageenan on colon inflammation could be connected to the various host immune responses to large or small amounts of oral carrageenan. The effect of carrageenan on the immune system of an organism under oral administration requires further investigations.

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