Carbohydrate Polymers 89 (2012) 914–919

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Structural and compositional characteristics of hybrid carrageenans from red algae *Chondracanthus chamissoi*

Peipei Wang[a], Xiaoliang Zhao[b], Youjing Lv[b], Miaomiao Li[a], Xin Liu[b], Guangsheng Li[a], Guangli Yu[a,b,*]

[a] Key Laboratory of Marine Drugs, Ministry of Education of China, School of Medicine and Pharmacy, Ocean University of China, Qingdao 266003, China
[b] Shandong Provincial Key Laboratory of Glycoscience and Glycoengineering, School of Medicine and Pharmacy, Ocean University of China, Qingdao 266003, China

ARTICLE INFO

Article history:
Received 15 February 2012
Received in revised form 30 March 2012
Accepted 13 April 2012
Available online 21 April 2012

Keywords:
Chondracanthus chamissoi
Hybrid carrageenan
Structural characterization
Oligosaccharide
ES-CID-MS/MS

ABSTRACT

Two polysaccharides CCC and CCH were extracted from red algae *Chondracanthus chamissoi* by cold water and hot water, respectively. Characterization of the structure by chemical and spectroscopic methods showed that CCC was a hybrid carrageenan composed of κ-carrageenan (35%) and ι-carrageenan (43%) along with its precursor μ-carrageenan (22%) and CCH was an ideal κ-carrageenan. Oligosaccharides from CCH and CCC were prepared and their structural sequences determined by ES-CID-MS/MS gave further insight into the structural characteristics of hybrid carrageenans from *C. chamissoi*. Some hybrid oligosaccharides, e.g., κ–κ, κ–μ and κ–ι, were obtained with mild acid hydrolysis of CCC. Then, a regular even-numbered oligosaccharides generated with reductive acid hydrolysis of CCH confirmed it an ideal κ-carrageenan.

1. Introduction

Carrageenans are highly sulfated galactans occurring in the cell walls of red algae (Rhodophyta) with a linear backbone of alternating 3-linked β-D-galactopyranose (G-unit) and 4-linked α-D-galactopyranose (D-unit). Classification of carrageenans is based on the occurrence of 3,6-anhydro form (A unit) in the 4-linked galactose residues and the pattern of sulfation. The most common types of carrageenans are traditionally called κ-, ι- and λ-carrageenan with different biose units of -[G4S-A]-, -[G4S-A2S]- and -[G2S-D2S6S]-, respectively (Knutsen, Myslabodski, Larsen, & Usov, 1997). However, native carrageenans are rarely in their uniformed or ideal form. The diversity of carrageenan is attributed to a mixed combination of different biose units or copolymeric chains. The most classical copolymers of carrageenan are those found in native κ- and ι-carrageenan chains that usually contain components of their biosynthetic precursors, e.g., μ- and ν-carrabiose with the biose unit of -[G4S-D6S]- and -[G4S-D2S6S]-, respectively (Jouanneau, Boulenguer, et al., 2010; Jouanneau, Guibet, et al., 2010). The structural heterogeneity of carrageenan composition, which depends on algal source, life stage, and extraction procedure (Pereira & van de Velde, 2011; Vandevelde, 2008) offers a wide range of physiochemical properties and biological activities, including gelling, thickening abilities (Vandevelde, 2008), antiviral (Lee, Takeshita, Hayashi, & Hayashi, 2011), antitumor activities (Zhou, Sheng, Yao, & Wang, 2006) and immunomodulatory activities (Stephanie, Eric, Sophie, Christian, & Yu, 2010).

Chondracanthus chamissoi is a benthic marine red algae and distributed from Paita, Peru (5°S) to Ancud, Chile (42°S). As an important source of income in Chile, *C. chamissoi* is widely used as raw material by the carrageenan industry and exported to many countries as edible seaweed (Buschmann, Correa, Westermeier, Hernández-González, & Norambuena, 2001). The harvesting of the red algae from natural populations has been and will be restricted by several authorities and thereby, stimulating the research in seaweed production by aquaculture (Bulboa, Macchiavello, Oliveira, & Fonck, 2005). Although earlier report by (Bixler, 1996) has shown polysaccharides from *C. chamissoi* contain κ and ι-carrageenan, their precise chemical structures are still to be elucidated. The aim of this study is structural characterization of two different carrageenans extracted from *C. chamissoi* in Chile.

Abbreviations: ES-MS, electrospray mass spectrometry; CID, collision-induced dissociation; GC–MS, gas chromatography–mass spectrometry; MMB, 4-methylmorpholine borane; CTMS, chlorotrimethylsilane; DP, degree of polymerization; Gal, galactose; anGal, 3,6-anhydrogalactose; A, 4-linked α-3,6-anhydrogalactose; Aol, 4-linked α-3,6-anhydrogalactitol; A2S, 4-linked 2-*O*-sulfated-α-3,6-anhydrogalactose; D, 4-linked α-D-galactopyranose; D6S, 4-linked 6-*O*-sulfated-α-D-galactose; G, 3-linked β-D-galactopyranose; G4S, 3-linked 4-*O*-sulfated-β-D-galactose.

* Corresponding author at: Shandong Provincial Key Laboratory of Glycoscience and Glycoengineering, School of Medicine and Pharmacy, Ocean University of China, Qingdao 266003, China. Tel.: +86 532 82031609; fax: +86 532 82033054.
E-mail address: glyu@ouc.edu.cn (G. Yu).

0144-8617/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2012.04.034

2. Experimental

2.1. Materials

The red seaweed *C. chamissoi* (Chile) was purchased from Kunshan Yihong Seaweed Co. Ltd. (Jiangsu, China). κ-Carrageenan standard, methylmorpholine-borane (MMB), $NaBD_4$ and ion-exchange resin Amberlite IR120 (H^+ form) were purchased from Sigma (Shanghai, China). Superdex Peptide HR column (1.0 cm × 30 cm) was purchased from Pharmacia Bioscience (Uppsala, Sweden). Fused-silica capillary columns DB-225 (30 m × 0.32 mm × 0.25 μm) and DB-225MS were purchased from J&W Scientific (Folsom, USA).

2.2. Extraction of polysaccharides

Extraction of polysaccharides was carried out as previously described by Yang et al. (2011). Briefly, the power of *C. chamissoi* was treated with 85% ethanol for 3 h at 80 °C (3 times) to remove lipids and the residue was dried. The residue was extracted with cold water for 3 h (3 times). The supernatant was combined, evaporated and precipitated with 4 volumes of ethanol. The precipitated polysaccharide (CCC) was dialyzed (7 kDa MWCO) against water and freeze-dried. The residue was further extracted with hot water at 80 °C using the above mentioned procedures and the polysaccharide CCH was obtained.

2.3. General analysis

Total sugar content was determined by the phenol–sulfuric acid method using galactose as standard (Cuesta, Suarez, Bessio, Ferreira, & Massaldi, 2003). The content of crude protein was determined by the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951). The anGal content was determined by the resorcinol method using fructose as standard (Yaphe & Arsenault, 1965). The sulfate content was determined by $BaCl_2$-Gelatin method (Dodgson & Price, 1962).

The purity and relative molecular weight (MW) of polysaccharides were determined by gel filtration chromatography on a PL aquagel OH column eluted with 0.2 mol/L Na_2SO_4 at a flow rate of 0.5 mL/min at 35 °C. The column was calibrated with Dextran standards, and the corrected regression equation was $y = -1.8773x + 24.376$ ($R^2 = 0.9997$).

The monosaccharide composition was determined quantitatively as their peracetylated alditols obtained by reductive hydrolysis followed by acetylation as described by Stevenson and Furneaux (1991). The resulting alditol acetates was analyzed by GC (Agilent HP5890 II, USA) using a fused-silica capillary column DB-225.

2.4. Spectroscopic analysis

FTIR spectra of polysaccharides CCC and CCH prepared as KBr pellets were recorded with a Nicolet Nexus 470 Thermo instrument. The ^{13}C NMR spectra were measured with a JNM-ECP 600 spectrometer at 25 °C. The polysaccharides were dissolved in D_2O and acetone-d_6 was used as an internal standard (Yang et al., 2011).

2.5. Desulfation and methylation

Desulfation of polysaccharides CCC and CCH was operated for three times continuously as described by Nagasawa, Inoue, and Kamata (1977). The sulfate content was determined as 5.6% and 6.7% by $BaCl_2$-Gelatin method, respectively. The desulfated polysaccharides were further purified on a Q-Sepharose FF column to remove small amount of sulfated polysaccharides, and the desulfated polysaccharides dsCCC and dsCCH were used for methylation analysis. Methylation was performed according to previous method by Hakomori (1964). The methylated polysaccharide was hydrolyzed and acetylated. Thereafter, partial methylated alditol acetates were analyzed by GC–MS equipped with a DB-225MS fused-silica capillary column.

2.6. Preparation and purification of oligosaccharides

Mild acid hydrolysis of κ-carrageenan standard (10 mg/mL) was carried out with 0.1 M H_2SO_4 at 60 °C for 1.5 h. Hydrolysis of polysaccharide CCC was extended to 3 h. Reductive hydrolysis of polysaccharide CCH was carried out with addition of 0.2 M MMB at 60 °C for 1.5 h. The reaction was terminated by neutralization with 2 M NaOH before analysis.

For oligosaccharide preparation, the hydrolysates were separated on a Superdex Peptide column eluted with 0.1 mol/L NH_4HCO_3 at a flow rate of 0.1 mL/min, using a refractive index detector (Yang et al., 2009).

2.7. ES-TOF-MS analysis

Negative-ion electrospray mass spectrometry (ES-MS) analysis on Micromass Q-Tof Ultima instruments (Waters, Manchester, UK) was performed for all oligosaccharides sequence analysis (Yu et al., 2006). Nitrogen was used as the desolvation and nebulizer gas at a flow rate of 500 L/h and 50 L/h, respectively. Source temperature was 80 °C and the desolvation temperature was 150 °C. Samples were dissolved in CH_3CN/2 mmol/L NH_4HCO_3 (1:1, v/v), typically at a concentration of 5–10 pmol/μL, of which 5 μL was loop-injected. Mobile phase (CH_3CN/2 mmol/L NH_4HCO_3, 1:1, v/v) was delivered by a syringe pump at a flow rate of 5 μL/min. Capillary voltage was maintained at 3 kV while cone voltage was 150 eV. For CID-MS/MS product-ion scanning, the collision energy was adjusted between 10 and 80 eV.

In order to get the oligosaccharide sequence, the deuterium reduction was carried out with $NaBD_4$ reagent as described by Yu et al. (2006).

3. Result and discussion

3.1. Extraction and composition analysis of polysaccharides

The cold water soluble polysaccharide CCC from *C. chamissoi* was the major component (25.8%). The yield of polysaccharide CCH extracted with 80 °C hot water was lower (5.2%) than that of CCC. Compared with the κ and ι-carrageenan standard, the chemical compositions of the two polysaccharides are listed in Table 1. Polysaccharides CCC and CCH showed a symmetric peak on a PL aquagel OH column, with average molecular weight of 465 kDa and 299 kDa, respectively. GC analysis showed that CCC and CCH mainly contained Gal and anGal. The ratio of Gal to anGal in CCC was 2.7, which was remarkably higher than κ and ι-carrageenan standard. The sulfate content of CCC was higher than κ- but lower than that of ι-carrageenan standard. Based on the GC analysis result and sulfate content, the structure character of CCC was different to ideal κ- or ι-carrageenans, it was a hybrid-sulfated galactan. CCH had a highly similar sulfate content and monosaccharide composition to κ-carrageenan standard.

3.2. FTIR and ^{13}C NMR analysis of polysaccharides

The FTIR spectra (Fig. 1) of CCC and CCH showed characteristic bands at 1260 cm^{-1} (S=O), 1072 cm^{-1} (Gal) and 930 cm^{-1} (anGal), but the absorption strength was different. The region around 800–850 cm^{-1} is used to identify the position of the sulfate