Carbohydrate Research 466 (2018) 1–10

Contents lists available at ScienceDirect

Carbohydrate Research

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

A sulfated heterorhamnan with novel structure isolated from the green alga *Monostroma angicava*

Xue Liu[a,c], Sujian Cao[a], Ling Qin[a], Meijia He[a], Hui Sun[a], Yajing Yang[a], Xiao Liu[a], Wenjun Mao[a,b,*]

[a] Key Laboratory of Marine Drugs of Ministry of Education, Shandong Provincial Key Laboratory of Glycoscience and Glycotechnology, School of Medicine and Pharmacy, Ocean University of China, Qingdao, 266003, China
[b] Laboratory for Marine Drugs and Bioproducts of Qingdao National Laboratory for Marine Science and Technology, Qingdao, 266237, China
[c] Biology Institute, Qilu University of Technology (Shandong Academy of Sciences), Jinan 250103, China

ARTICLE INFO

Keywords:
Green alga
Sulfated heterorhamnan
Structural characteristics
Oligosaccharide
NMR
ES-CID MS/MS*Chemical compounds studied in this article:*
Ammonium bicarbonate (PubChem CID: 14013)
Barium rhodizonic acid (PubChem CID: 45048930)
Deuterium oxide (PubChem CID: 24602)
Dimethyl sulfoxide (PubChem CID: 679)
D-glucuronic acid (PubChem CID: 94715)
Iodomethane (PubChem CID: 6328)
1-Phenyl-3-methyl-5-pyrazolone (PubChem CID: 90474051)
Pullunan (PubChem CID: 92024139)
Rhamnose (PubChem CID: 25310)
Trifluoroacetic acid (PubChem CID: 6422)

ABSTRACT

A sulfated polysaccharide, designated MAP2, was isolated from *Monostroma angicava* by water extraction, anion-exchange and size-exclusion chromatography. The structural characteristics of MAP2 were investigated by chemical and spectroscopic methods, including methylation analysis, one- and two-dimensional nuclear magnetic resonance and electrospray mass spectrometry with collision-induced dissociation spectroscopic analyses. The results showed that MAP2 was primarily composed of rhamnose with small amounts of xylose, glucuronic acid and glucose. The molecular weight of MAP2 was estimated to be about 671 kDa. The backbone of MAP2 was mainly constituted by 3-linked, 2-linked-á-L-rhamnose residues. Sulfate substitutions were at C-2/C-4 of 3-linked-á-L-rhamnose and C-3/C-4 of 2-linked-á-L-rhamnose residues. The branches consisted of 3-linked and 2-linked-á-L-rhamnose with monosulfate/unsulfate, as well as small amounts of β-D-GlcA-(1→ and β-D-GlcA (2SO_4)-(1 →. Minor amounts of →4)-D-Glc*p*-(1→ and β-D-Xyl*p* (4SO_4)-(1→ might also be existent in MAP2. The investigation demonstrated that MAP2 was a novel sulfated rhamnan distinguishing from other algal sulfated rhamnans.

1. Introduction

Algae have been used widely for centuries in the world as food and the production of valuable chemicals, and polysaccharides are the major chemical compound [1,2]. So far, many sulfated polysaccharides from red and brown algae have been isolated and characterized [3–7]. However, a few limited reports on structure of the sulfated polysaccharides from green algae have been demonstrated. It is worthy to note that some green algae produce specific sulfated polysaccharides which are mainly composed of α-L-rhamnose moiety. A α-L-rhamnose is a rare monosaccharide in human and animals, whereas it is widely distributed in plants and microorganism as component of glycosides or polysaccharides [8,9]. However, distribution of a polymer composed of large amounts of α-L-rhamnose, so-called “rhamnan” is quite limited in the nature [10]. Some investigations demonstrated that the sulfated rhamnans from green algae had high anticoagulant, antiviral, anticancer and immunomodulatory activities [11–18]. With today's interest in new sources of chemicals and polymers, the sulfated rhamnans from green algae represent a potential source to be explored.

The green alga *M. angicava,* a major species of the genus *Monostroma*, grows in upper part intertidal zone and is broadly distributed through the World's seas. In the present work, a sulfated heterorhamnan with novel structure from *M. angicava* was extracted using boiling distilled water and subsequently purified using ion-exchange and gel filtration chromatography. The detailed structure of the sulfated rhamnan was characterized by a combination of chemical and spectroscopic methods.

* Corresponding author. Key Laboratory of Marine Drugs of Ministry of Education, Shandong Provincial Key Laboratory of Glycoscience and Glycotechnology, School of Medicine and Pharmacy, Ocean University of China, Qingdao, 266003, China.
E-mail address: wenjunmqd@hotmail.com (W. Mao).

https://doi.org/10.1016/j.carres.2018.06.010
Received 30 April 2018; Received in revised form 4 June 2018; Accepted 25 June 2018
Available online 26 June 2018

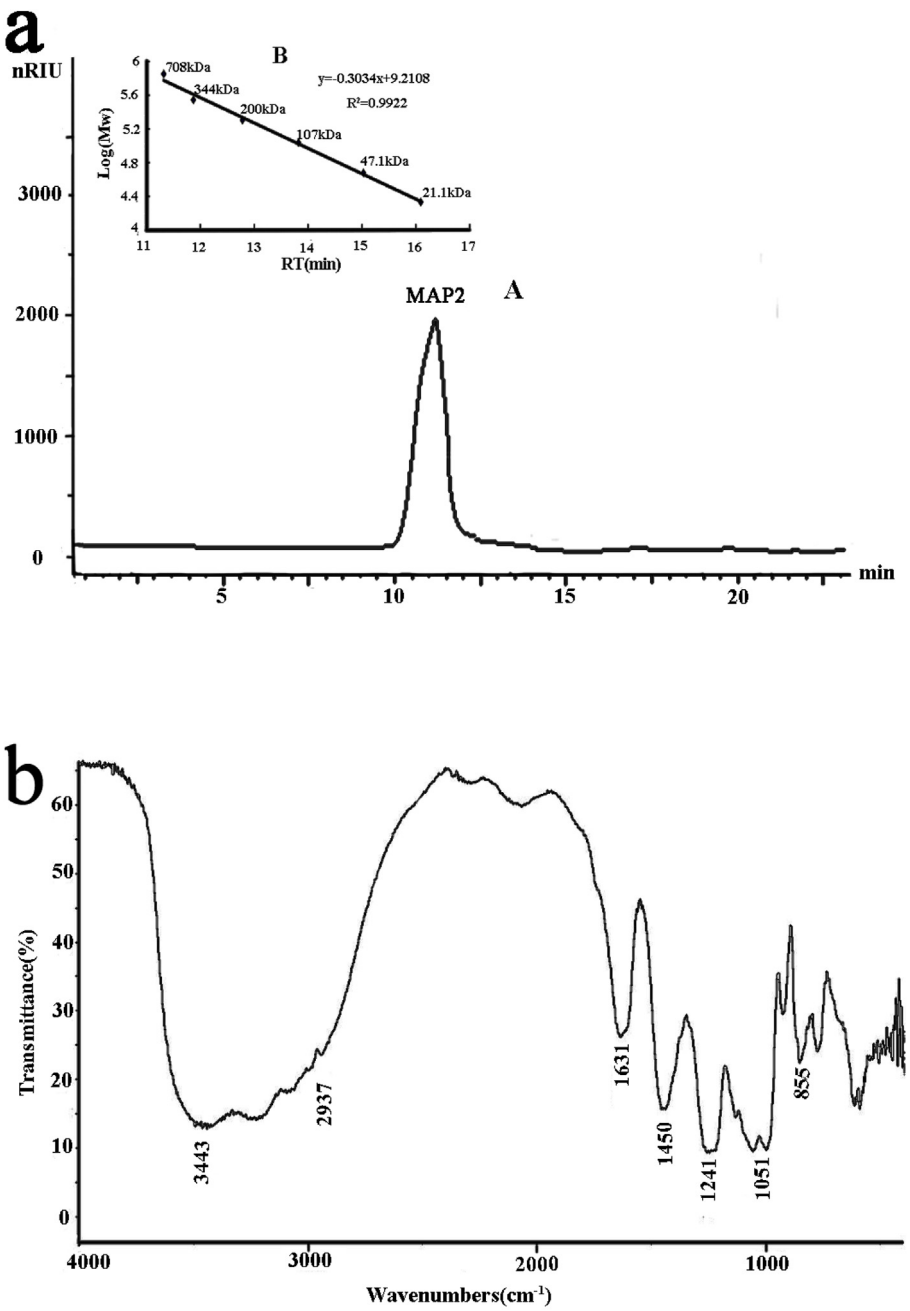

Fig. 1. HPGPC chromatogram and IR spectrum of MAP2. (a) HPGPC chromatogram of MAP2 on a Shodex OHpak SB-804 HQ column (A) and the standard curve of molecular weight (B); (b) IR spectrum of MAP2.

2. Results and discussion

2.1. Composition of the sulfated polysaccharide MAP2

The crude polysaccharide was extracted from *M. angicava* using boiling water, and further fractionated using a Q Sepharose Fast Flow column and Sephacryl S-400/HR column. A polysaccharide fraction, designed MAP2, was obtained. The yield of MAP2 from crude polysaccharide (w/w) was about 20.92%. As showed in Fig. 1a, MAP2 appeared as a single and symmetrical peak in the high performance gel permeation chromatography (HPGPC), indicating its purity and corresponding to an average molecular weight of about 671 kDa. MAP2 contained 29.35% sulfate ester, 8.77% uronic acid, and no protein was detected. High performance liquid chromatography (HPLC) analysis showed that MAP2 was mainly composed of rhamnose (82.79%) with small amounts of xylose (6.09%), glucose (2.38%) and glucuronic acid (8.74%). Thus, MAP2 was a sulfated heterorhamnan. Absolute configuration analysis by HPLC showed that the rhamnose (29.44 min) in MAP2 was L-configuration, whereas glucuronic acid (19.12 min), xylose (19.88 min) and glucose (16.95 min) were D-configuration, because their retention times were in agreement with retention times of the derivatives of the response standards, but differences from retention times of the corresponding derivatives of the opposite enantiomers (Supplemental Fig.1).

Fourier-transform infrared (FTIR) spectrum of MAP2 demonstrated several bands corresponding to sulfate ester (Fig. 1b): the peaks at 855 cm^{-1} and 1241 cm^{-1} derived from stretching vibration of C–O–S of sulfate in axial position and stretching vibration of S–O of sulfate, respectively. The intense and broad band at 3443 cm^{-1} was due to the stretch vibration of hydroxyl groups, and the signal at 2937 cm^{-1} was