International Journal of Biological Macromolecules 67 (2014) 336–342

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

A study of neuroprotective and antioxidant activities of heteropolysaccharides from six *Sargassum* species

Weihua Jin[a,b,c], Wenjing Zhang[a,b], Jing Wang[a,c], Jianting Yao[a], Enyi Xie[d], Dongchao Liu[d], Delin Duan[a], Quanbin Zhang[a,*]

[a] *Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, PR China*
[b] *University of Chinese Academy of Sciences, Beijing 100049, PR China*
[c] *Nantong Branch, Institute of Oceanology, Chinese Academy of Sciences, Nantong 226006, Jiangsu, PR China*
[d] *Fisheries College, Guangdong Ocean University, Zhanjiang 524025, PR China*

ARTICLE INFO

Article history:
Received 15 January 2014
Received in revised form 17 March 2014
Accepted 19 March 2014
Available online 27 March 2014

Keywords:
Neuroprotective activity
Antioxidant activity
Sargassum

ABSTRACT

Heteropolysaccharides were extracted from *Sargassum integerrimum* (S.I), *Sargassum maclurei* (S.M), *Sargassum naozhouense* (S.N), *Spiraea thunbergii* (S.T), *Sargassum hemiphyllum* (S.H) and *Sargassum fusiforme* (S.F), and their neuroprotective effects and antioxidant activities were investigated. It showed that S.I, S.N, S.T and S.F exhibited neuroprotective activities, whereas S.H and S.M did not. For this reason, they were separated by anion-exchange chromatography. It was apparent that the fraction 2 represented the principal difference between the active and non-active compounds. However, it did not correlate with neuroprotective effect. In addition, the results on antioxidant activities showed that the hydroxyl-radical scavenging effect contribute to the neuroprotective effect of S.T and S.N, and the DPPH-radical scavenging effect and reducing power contribute to S.T, S.F and S.I. However, the superoxide-radical scavenging effect did not correlate with neuroprotective activity. The conclusion was that the neuroprotective activity of the family of compounds investigated depended on a variety of factors.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Sulfated polysaccharides are recognized to possess various biological activities, including anticoagulant, antithrombotic, antiviral, antioxidant, antitumor, anti-inflammatory and immune-modulating activities. With the exception of certain mammalian polysaccharides, the fucoidans and sulfated galactans of algae are the most well—studied sulfated polysaccharides. In terms of total biomass, they are more abundant than glycosaminoglycans.

Fucoidans are a family of sulfated polysaccharides found primarily in the cell-wall matrix of various brown seaweed species [1–6]. They show anticoagulant, antitumor and immunomodulatory activities that correlate with their chemical structures. The detailed chemical structures of these polysaccharides differ from species to species and also vary with extraction and/or purification methods, the harvest time and the location of algae. A sulfated polysaccharide isolated from a given species of brown algae might be a mixture of structurally different polysaccharides. Thus, the structure–activity relationship of fucoidans is still an unresolved problem.

Fucoidans are complex and structurally heterogeneous polysaccharides. Fractionation of fucoidans by ion-exchange chromatography produces many fractions. Brown algae contain at least two types of sulfated fucose-containing polysaccharides [7,8]. In addition to typical fucoidans, which contain substantial amounts of fucose and sulfate together with other minor monosaccharides (technically, the typical fucoidans are sulfated fucans) and have been extensively investigated, forms of heteropolysaccharides have also been found in brown algae. They contain small amounts of fucose and sulfate together with other major monosaccharides (in technical terms, these compounds are fucomannoglucuronans). Because this type of heteropolysaccharides is very complex, some reports [8–12] have addressed their structural features and their biological activities.

Unlike *Fucus* and *Ascophyllum*, in which fucoidans are the dominant sulfated polysaccharides, *Sargassum* is rich in heteropolysaccharides with a low fucose and sulfate content. To investigate the chemistry and bioactivities of heteropolysaccharides, we performed a comparative study of the chemical constituents and neuroprotective and antioxidant activities of

* Corresponding author. Tel.:+86 532 82898703/+86 013687638267; fax: +86 532 82898703.
E-mail addresses: jinweihua09@mails.ucas.ac.cn (W. Jin), qbzhang@ms.qdio.ac.cn (Q. Zhang).

http://dx.doi.org/10.1016/j.ijbiomac.2014.03.031
0141-8130/© 2014 Elsevier B.V. All rights reserved.

heteropolysaccharides from six Chinese *Sargassum* species, including *Sargassum integerrimum*, *Sargassum maclurei Setch*, *Sargassum naozhouense*, *Spiraea thunbergii*, *Sargassum hemiphyllum*, and *Sargassum fusiforme* (*Hizikia fusiforme*). Reports of heteropolysaccharides extracted from the first-named three species are scarce. It was reported [13] that a hot-water extract from *S. hemiphyllum* showed antioxidant and immune-stimulating activities. The extract consisted of water (10.1%), crude protein (38.5%), crude fat (8.4%), ash (12.2%) and total carbohydrate (30.8%). Another study [14] showed that an aqueous extract from *S. hemiphyllum* had anti-inflammatory activity. The monosaccharides found in this polysaccharide extract included myo-inositol, sorbitol, fucose, galactosamine, galactose, glucose and mannose. The most abundant monosaccharide in the extract was fucose. To our knowledge, however, no other reports have addressed the structural characteristics and chemical composition of *S. hemiphyllum*. *S. fusiforme* (*H. fusiforme*) is a traditional health food and Chinese herbal medicine. The use of *S. fusiforme* as a traditional Chinese herbal medicine has been documented in Shennong's Herbal and Compendium of Materia Medica. *S. fusiforme* has a variety of biological activities, including anticoagulant, antitumor, and immunomodulation activity. Li [10] reported that fucoidan extracted from *S. fusiforme* consisted of →2) α-D-Man (1→ and →4) β-D-GlcA (1→ in alternation, with an occasional occurrence of →4) β-D-Gal (1→ in the main chain. The branch points were at C-3 of →2) α-D-Man (1→, C-2 of →4) β-D-Gal (1→ and C-2 of →6) β-D-Gal (1→. Approximately 2/3 of the fucoses were at the nonreducing ends, and 1 → 4, 1 → 3 and 1 → 2 glycosidic linkages were located to the left of the fucoses. Approximately 2/3 of the xyloses were at the nonreducing ends, and 1 → 4 glycosidic linkages were located to the left of the xyloses. The sulfate groups were at C-6 of →2, 3) α-D-Man (1→, C-4 and C-6 of →2) α-D-Man (1→, C-3 of →6) β-D-Gal (1→, C-2, C-3 or C-4 the fucose, whereas several fucoses had two sulfate groups. Several structural features were confirmed by ESI–CID–MS/MS [12].

As the population ages, the prevalence of neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's (PD) is increasing. PD, the second most common neurodegenerative disorder, affected 2% of the population aged greater than 60 years. Parkinson's disease is a neurodegenerative disorder of uncertain pathogenesis characterized by a loss of dopaminergic neurons in the substantia nigra pars compacta and can be modeled by the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Oxidative stress may contribute to MPTP- and Parkinson's disease-related neurodegeneration. In a previous study [15], we showed that fucoidan protected brain function in a MPTP-induced neurotoxicity model of Parkinson's disease through its antioxidative activity. Although the relatively large size of fucoidan prevents penetration of the blood–brain barrier, systemic administration was effective in maintaining neuronal function in this mouse model. In addition, it was shown [16] that fucoidan exhibited a neuroprotective effect on H_2O_2-induced apoptosis in PC12 cells via activation of the PI3K/Akt pathway.

To the best of our (admittedly limited) knowledge, no other studies have addressed the effectiveness of algal heteropolysaccharides in the treatment of Parkinson's disease. In this study, we investigated the neuroprotective effects and antioxidant activities of heteropolysaccharides from *Sargassum* species.

2. Materials and methods

S. integerrimum, *S. maclurei*, *S. naozhouense*, *S. hemiphyllum*, and *S. fusiforme* were collected in Zhanjiang, Guangdong Province. *S. thunbergii* was collected in Rongcheng, Shandong Province. The fresh seaweed was washed with seawater and sun dried. These seaweeds were authenticated by Prof. Delin Duan.

2.1. Extraction of heteropolysaccharides

Algae (100 g) were cut into pieces. The heteropolysaccharides were extracted from the respective algae with water (2 L) at 100 °C for 4 h. The extracting solutions were filtered with celite and concentrated. Further elimination of alginic acid was achieved by using 20% ethanol with $MgCl_2$ (0.05 M L^{-1}). After removing the alginic acid, the supernatant fluid was dialyzed with running water for one day and distilled water for one day. Finally, the dialysate was concentrated and the heteropolysaccharide was obtained by ethanol precipitation (Final concentration of ethanol was 80%).

2.2. Anion-exchange chromatography

The heteropolysaccharides (1 g) were fractionated by anion-exchange chromatography on a DEAE-Bio Gelagrose FF (2.6 cm × 30 cm). Firstly, they were balanced with water (named fraction 1) and then separated with gradient elution from 0 to 2 M NaCl (1 L, respectively) at a flow rate 7.4 mL min^{-1}. It was collected every 1.5 min per tube by automatic sampling instrument. And they were detected by phenol–sulfuric acid method at the absorbance of 485 nm. The tube 30th to tube 40th was marked as fraction 2 while the tube 41th to 100th was marked as fraction 3. Fraction 4 was collected from 101th to the last tube.

2.3. Compositional analysis

The sulfate content was analyzed by ion chromatography on Shodex IC SI-52 4E column (4.0 × 250 mm) eluted with 3.6 mM Na_2CO_3 at a flow rate of 0.8 mL min^{-1} at 45 °C. Ratio of monosaccharides and the content of fucose were analyzed following the methods described by Zhang et al. [17]. Briefly speaking, heteropolysaccharides (10 mg mL^{-1}) were hydrolyzed by trifluoroacetic acid (2 M) under nitrogen atmosphere for 4 h at 110 °C. Then the hydrolyzed mixture was neutralized to pH 7 with sodium hydroxide. Thus obtained monosaccharides were converted into its 1-phenyl-3-methyl-5-pyrazolone derivatives and separated by HPLC chromatography on YMC Pack ODS AQ column (4.6 × 250 mm). Uronic acid was determined by a modified carbazole method [18].

2.4. IR spectroscopy

IR spectra of pellet preparations of the fractions have been recorded in the region 400–4000 cm^{-1} on a Nicolet-360 FTIR spectrometer (36 scans, at a resolution of 6 cm^{-1}).

2.5. Cell culture and MTT assay

MES 23.5 cells were maintained in Dulbecco's modified Eagle's medium (Gibco Industries, Inc. (Auckland, New Zealand)), supplemented with 5% fetal bovine serum (Gibco Industries, Inc. (Auckland, New Zealand)), 100 units mL^{-1} penicillin-streptomycin (Gibco Industries, Inc. (Auckland, New Zealand)) in an atmosphere of 5% CO_2 at 37 °C. Then, MES 23.5 cells (200 μL) were seeded in a 96-well plate at a density of 2×10^5 cells/wells for 24 h prior to experimentation. Subsequently, they were divided into three groups: (1) Control group: control cells were treated in a serum-free medium for 24 h. (2): 6-hydroxydopamine (6-OHDA) group: cells were treated by 6-OHDA (100 μM) (Sigma-Aldrich (St Louis, MO, USA)) in a serum-free medium for 24 h; (3): experimental groups: cells were treated by 6-OHDA (100 μM) and various heteropolysaccharides at the different concentrations (1 mg mL^{-1} and 0.1 mg mL^{-1}) in a serum-free medium for 24 h. Following the removal of medium from the wells, 10 μL of MTT (5 mg mL^{-1} suspended in 0.01 M PBS) was added to each well. After 4 h of

Download English Version:

https://daneshyari.com/en/article/8333129

Download Persian Version:

https://daneshyari.com/article/8333129

Daneshyari.com