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The structural features of the sulfated heteropolysaccharide (ST-1) from *Sargassum thunbergii* and its neuroprotective activities

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ABSTRACT

Polysaccharide (ST) was prepared from *Sargassum thunbergii* using hot water. Two fractions (ST-1 and ST-2) were prepared using anion exchange chromatography. One desulfated polysaccharide (ST-1-DS) was also prepared. Electrospray ionization mass spectrometry (ESI–MS) performed on ST-1-DS showed that the desulfated polysaccharides contained methyl glycosides of mono-sulfated and di-sulfated galactofucooligosaccharides. This result suggested that ST-1 might contain sulfated galactofucan, which consists of a backbone of alternating $(Gal)_n$ and $(Fuc)_n$ and sulfated randomly on Gal and mainly on C-2 in Fuc. In addition, ST-1 was degraded in 1 M sulfuric acid. The solution was centrifuged, and the supernatant was concentrated and precipitated in ethanol to obtain the precipitate (ST-1-P). ST-1-P was then separated using gel chromatography and anion exchange chromatography to obtain the oligomers. ESI–MS spectra of oligomers indicated that ST-1 mostly contained sulfated glucuronomannan and fucoglucuronan. ESI–MS with collision-induced dissociation tandem mass spectrometry (ESI-CID-MS/MS) suggested that glucuronomannan contained alternating 2-linked Man and 4-linked GlcA, while fucoglucuronan contained 4-linked glucuronan with branched Fuc at C-3. Finally, the neuroprotective activities of ST, ST-1, ST-2 and MIX (a mixture of ST-1 and ST-2) were determined. ST showed the most neuroprotective activity, which indicated that ST might be a good candidate for curing neurodegenerative diseases.

1. Introduction

Sargassum thunbergii is a brown alga species that is widely distributed in the Sea of Japan and the China Sea. It has been reported that *S. thunbergii* contains many bioactive compounds, such as indole derivatives, isopentadiene, phlorotannins, and flavonoids [1–6].

Few reports have focused on the water extraction method, especially for the polysaccharides of *S. thunbergii*. Itoh et al. [7,8] reported the antitumour activities and immunological properties of polysaccharides prepared from *S. thunbergii*. Park et al. [9] found that enzymatic extracts had a reactive oxygen scavenging effect. Recently, our group reported [10] that polysaccharides from *S. thunbergii* have neuroprotective and antioxidant activities. However, few studies have investigated the structures of polysaccharides from *S. thunbergii*. Luo et al. [11] reported that 3-linked fucose, 3-linked xylose and 3-linked galactose form the major components of the heteropolysaccharide from *S. thunbergii.* In addition, Yuan et al. [12] indicated that a polysaccharide (STP-II) from *S. thunbergii* showed antioxidant and inhibitory activity in vitro against human colon cancer Caco-2 cells. To the best of our knowledge, only Luo et al. [11], Zhuang et al. [13] and our group [10] have used chemical and spectral analysis to show that the polysaccharides consist mainly of fucoidan or L-fucan. Ren et al. [14] used microwave-assisted extraction to obtain polysaccharides, and they showed that the polysaccharides exhibited antioxidant and hypoglycaemic activities. Hu et al. [15] and Ou et al. [16] showed that the fucoidan-like polysaccharide STPC2 had anti-angiogenic activity.

As the population ages, the prevalence of neurodegenera-tive diseases such as Alzheimer's disease (AD) and Parkinson's (PD) is increasing. PD, the second most common neurodegener-ative disorder, affected 2% of the population aged greater than 60 years. Parkinson's disease is a neurodegenerative disorder ofuncertain pathogenesis characterized by a loss of dopaminergicneurons in the substantia nigra pars compacta and can be modeledby the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine(MPTP) [10]. In the previous studies, it was found that polysaccharides from *Sargassum crassifolium* had antioxidant and neuroprotective effects [17]. In addition, polysaccharides from *Turbinaria decurrens* had a

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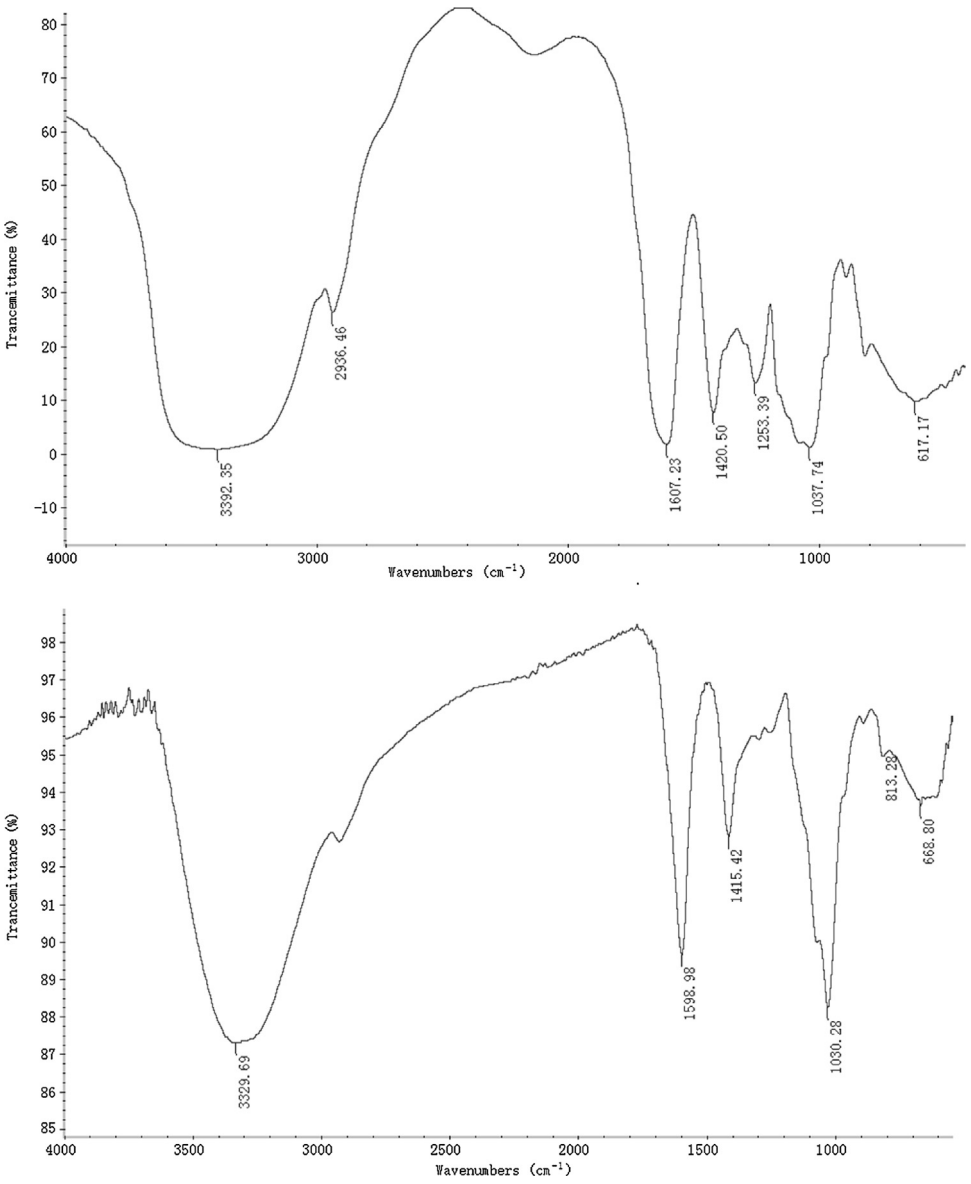

Fig. 1. IR spectra of ST-1 (top) and ST-1-DS (bottom).

neuroprotective effect in MPTP intoxicated Parkinsonic mice [18]. Moreover, polysaccharides from *Saccharina japonica* had a neuroprotective effect against dopaminergic neuron death [19].

In this study, we investigated the structural features of ST-1. We also studied polysaccharides from the brown alga *S. thunbergii* to search for natural neuroprotective agents.

2. Materials and methods

2.1. Materials

The brown alga *Sargassum thunbergii* was collected in Qingdao, China, on May 28, 2014. The L-fucose, D-galactose, D-mannose, D-glucuronic acid, L-rhamnose monohydrate, D-xylose and D-glucose standards were purchased from Sigma. The 3-methyl-1-phenyl-2-pyrazolin-5-one (PMP) (99%) was obtained from Aldrich Chemistry.

2.2. Preparation and purification of polysaccharides

Algae (100 g) were cut into pieces and treated with 85% ethanol three times to remove pigment. Crude polysaccharide was extracted from the residual material using hot water (3 L) for 4 h. The extract solution was filtered with Celite and concentrated. Further elimination of alginate was achieved using 20% ethanol containing 0.05 M L^{-1} $MgCl_2$. After removing the alginate, the supernatant was ultra-filtered. Finally, the dialysate was concentrated and crude polysaccharide was obtained using ethanol precipitation. We referred to this crude polysaccharide as "ST."

We performed anion exchange chromatography on crude polysaccharide (ST) (6 g) using a DEAE-Bio Gel Agarose FF gel (6 cm × 40 cm) with elution by 0.5 M (5 L) (ST-1) and 2 M (5 L) (ST-2). The polysaccharides were then dialyzed, concentrated and precipitated in ethanol. The yields of ST, ST-1 and ST-2 were 1.4%, 23.3% and 25.5%, respectively.

2.3. Preparation of desulfated polysaccharides and oligosaccharides

The desulfated polysaccharide was prepared according to a previously described method [20]. ST-1 (1 g) was dissolved in distilled water (100 mL) and mixed with cationic resin (H^+) (25 g) for 3 h. After filtration, the solution was neutralized with pyridinium and