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The structure-activity relationship between polysaccharides from *Sargassum thunbergii* and anti-tumor activity

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ABSTRACT

Polysaccharides derived from *Sargassum thunbergii* were prepared to investigate the structure-activity relationship between polysaccharides and anti-tumor activity *in vitro*. Many factors were examined. Overall, STW (polysaccharide extracted by hot water) had the best activity, followed by STJ (polysaccharide extracted by dilute alkali), and then STA (polysaccharide extracted by dilute acid). Location of algae had no effect at 500 μg/mL and 1000 μg/mL, while STW-QD (algae collected from Qingdao, China) had the best activity, followed by STW-WZ (algae collected from Wenzhou, China) and STW-LJ (algae collected from Lianjiang, China) and then STW-DL (algae collected from Dalian, China) and STW-RC (algae collected from Rongcheng, China) at 250 μg/mL. Moreover, molecular weight had no effect at 1000 μg/mL, while higher molecular weights were associated with better activities at 250 μg/mL and 500 μg/mL. Sulfate content had no effect at 1000 μg/mL, while anti-tumor activities decreased accompanying with the changes of sulfate content. Uronic acid content was an important factor influencing activity. The fractions of STW showed little anti-tumor activity; however, the mixture of the fractions of STW showed approximately 60% inhibition. Overall, these findings suggested that the anti-tumor activity of polysaccharides required multilateral cooperation and that some of the effective components were lost.

1. Introduction

The brown alga, *Sargassum thunbergii*, is a common intertidal seaweed species along the northwestern Pacific coast, which is characterized by a warm-temperate and humid maritime climate [1]. This alga is commonly used as bait and a component of artificial *Sargassum* beds due to its wide ecological amplitude and high economic and ecological value [1–3]. Increased demand for *S. thunbergii* in response to the rapid development of sea cucumber aquaculture in China has resulted in the depletion of natural populations along the coast of China. Thus, a large-scale aquaculture of *S. thunbergii* is under way [4].

Seaweeds are known for being rich in polysaccharides, minerals and certain vitamins, but they also contain bioactive substances including polysaccharides, proteins, lipids, polyphenols, isopentadiene, phlorotannins, and quinone, which have anti-bacterial, anti-oxidant, anti-coagulant, and neuroprotective effects [5–16]. Many studies have investigated the crude extracts of *S. thunbergii*. For example, Park et al. [17] reported that the enzymatic extracts of *S. thunbergii* had reactive oxygen scavenging effects. Moreover, Lee et al. [18] showed that the 70% ethanol extract from *S. thunbergii* had anti-oxidant and anti-neuroinflammotory effects. Additionally, Kim *et al.* [19] showed that the methanol extract from *S. thunbergii* had anti-oxidant and matrix metalloproteinase inhibitory effects. However, few studies have investigated polysaccharides from *S. thunbergii*. Itoh et al. [20,21] and Zhuang et al. [22] found that polysaccharides (GIV-A and GIV-B) from *S. thunbergii* had anti-tumor activities; however, both of the active compositions GIV-A and GIV-B were sulfate polysaccharides, which were composed of fucose, uronic acid and sulfate. Our group [23] reported that a crude polysaccharide from *S. thunbergii* possessed neuroprotective and antioxidant activities. Yuan et al. [24] recently reported that polysaccharides (STP-II) from *S. thunbergii* had good antioxidant and inhibitory activities against human colon cancer Caco-2 cells

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in vitro. However, STP-II primarily consisted of fucose, xylose, galactose, glucose and glucuronic acid. Structural analysis [11] indicated that 3-linked fucose, 3-linked xylose and 3-linked galactose formed the main-chain structure of STP-II, and that the branch ratios were 17.5%.

Active compounds from marine organism have been the object of many investigations during the search for natural anti-tumor compounds. It has been reported that fucose rich sulfated polysaccharides isolated from brown seaweeds exhibited anti-tumor activity, which is one of the most important biological activities of seaweeds [25]. Anastyuk et al. [26] reported that a crude polysaccharide from the brown alga *Fucus evanescens* exerted anticancer activities in the human malignant melanoma cell lines, SK-MEL-28 and SK-MEL-5, but that its low molecular weight fragments exhibited no activity toward cell proliferation in either cell line. Conversely, some studies suggested that low molecular weight polysaccharides have more biological activities than native compounds. The pharmacological effects of polysaccharides vary with their molecular weight, which is generally classified as low (<10 kDa), medium (10–10,000 kDa), or high (>10,000 kDa) [5,27]. Teruya et al. [28] compared two polysaccharides, a crude polysaccharide from *Cladosiphon okamuranus* and its sulfate derivatives, and found that the oversulfated polysaccharide reduced the proliferation of U937 cells in a dose-dependent manner, but the anti-tumor activity of the native form was weak. Vishchuk et al. [29] reported that sulfated polysaccharides (the purified polysaccharides Up-F2 and Sj-F2) were fractionated upon DEAE-cellulose chromatography of the crude polysaccharide extracted by acid from *Undaria pinnatifida* and *Saccharina japonica*. Further, they found that the inhibitory activities of Up-F2 and Sj-F2 toward human ductal carcinoma cell T-47D were approximately 60% and 25%, respectively, while they both showed approximately 60% inhibitory activity toward human malignant melanoma cell SK-MEL-28 at 0.8 mg/mL.

Although many studies have investigated sulfated polysaccharides from brown seaweeds, there have been no distinct and reliable conclusions regarding the chemical structures responsible for the specific biological activities of sulfated polysaccharides. This is because the chemical structures of polysaccharides used in biological experiments were not fully characterized in most cases. In this study, many factors, including the extraction methods, locations of algae, molecular weight, sulfate, uronic acid (UA) content, and fractions, were investigated to clarify the structure-activity relationship between polysaccharides from *S. thunbergii* and their anti-tumor activity.

2. Materials and methods

2.1. Preparation of polysaccharides

S. thunbergii were collected from various locations in Dalian (July of 2015), Rongcheng (May of 2015), Qingdao (June of 2015), Wenzhou (May of 2015) and Lianjiang (May of 2015). Other algae were collected from Dongtou, China, in July of 2014. The polysaccharides were extracted from *S. thunbergii* as previously described [30].

Briefly, the dried algae were cut into pieces and treated with 85% ethanol to remove pigment three times. Crude polysaccharides were then extracted from the residual material with hot water (3L) for 4 h. The extract solution was filtered with Celite and concentrated. Further elimination of alginate was achieved using 20% ethanol with $MgCl_2$ (0.05 M/L). After removing the alginate, the supernatant fluid was ultra-filtered. Finally, the dialysate was concentrated, and crude polysaccharide was obtained by ethanol precipitation and named STW (yield, 2.57%). The polysaccharides from different locations of *S. thunbergii* were named STW-DL (Dalian; yield, 1.81%), STW-RC (Rongcheng; yield, 1.65%), STW-QD (Qingdao; yield, 1.30%), STW-WZ (Wenzhou; yield, 1.82%) and STW-LJ (Lianjiang; yield, 1.85%), respectively.

Crude polysaccharide was extracted from the residual material with 0.1 M HCl (2 L) at room temperature for 3 h. The extract solution was filtered with Celite, ultra-filtered and concentrated. Finally, the crude polysaccharide was obtained by ethanol precipitation and named STA (yield, 2.02%).

The polysaccharides were extracted from the residual material by diluted sodium carbonate for 2 h. After removing the alginate, the supernatant was concentrated and dialyzed, after which crude polysaccharide was obtained by ethanol precipitation and named STJ (yield, 2.74%).

2.2. Preparation of low molecular weight polysaccharides

The crude polysaccharide STW was degraded using hydrogen dioxide and ascorbic acid to obtain low molecular weight polysaccharides, as previously described [30]. Briefly, crude polysaccharide (1 g) was dissolved in water (100 mL). Ascorbic acid (0.25 g) and hydrogen dioxide (0.15 mL) were then added, after which the solution was stirred for 2 h at room temperature. The degraded polysaccharide STW-D1 (yield, 83.58%) was obtained after ultrafiltration, concentration and lyophilization. STW-D2 (yield, 78.00%) was obtained by ascorbic acid (0.5 g) and hydrogen dioxide (0.3 mL), and STW-D3 (yield, 68.33%) was obtained by ascorbic acid (0.5 g) and hydrogen dioxide (0.3 mL) at 60 °C.

2.3. Anion-exchange chromatography

STW was separated by anion-exchange chromatography on a DEAE-Bio Gel agarose FF (5 cm × 60 cm) column with water and a liner gradient solution of NaCl (0–2 mol/L) at a flow rate of 10 mL/min. The compound was detected by the phenol-sulfuric acid method, after which STW was divided into three fractions, STW-1, STW-2 and STW-3. The mixture (MIX) was obtained by combing STW-1 (yield, 13.01%), STW-2 (yield, 53.88%) and STW-3 (yield, 14.38%) with a mass ratio of 1.00: 4.14: 1.11, which was the ratio of the yields.

2.4. Functional groups

The desulfation of STW was performed according to the method described by Nagasawa et al., with slight modification [31]. To accomplish this, desulfation was conducted using its pyridinium salt. Briefly, the sample was dissolved in distilled water (10 mL), then mixed with cationic resin for 3 h. After filtration, the solution was neutralized with pyridinium and lyophilized. The polysaccharide was subsequently dissolved in 20 mL of a 9:1 ratio of dimethyl sulfoxide: methanol (v:v) at 80 °C for 5 h. The desulfated solution was then dialyzed and lyophilized to give desulfated products (STW-DS) (yield, 52.00%).

Esterification reaction was accomplished using sulfur trioxide-pyridine. The polysaccharide (1 g) with sulfur trioxide-pyridine (5 g) was added into dimethylformamide (DMF) at 60 °C for 24 h, after which the solution was neutralized, dialyzed, concentrated and lyophilized. The obtained polysaccharide was named STW-S (yield, 173.31%).

The carboxyl reduction was performed according to a previous study [32]. Briefly, STW (10 mg) was dissolved in distilled water (10 mL), after which 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide (EDC) was added (10 mg). The solution was subsequently stirred at room temperature for 1 h under constant pH (approximately 4.8), which was maintained by the addition of 0.1 M HCl. Freshly prepared 2 M sodium borohydride (1 mL) was then added twice during the next 2 h at 50 °C. Later, the reac-

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