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Chemical characterization of polysaccharides from three seaweed species and their potential biological activities

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Introduction

Many kinds of seaweeds have been consumed as foodstuff in Japan, Korea, China and parts of Europe, and they are rich in many bioactive compounds which is beneficial to human health [1] such as fucoidan, agar and carrageenan, and are also used extensively in industry [2].

In recent years, medical potential of seaweed polysaccharides has drawn more and more attention of scientists. For example, anti-allergic, antitumor, antivirus, and anticoagulant biological activities have been found in seaweed polysaccharides. Their different structures may relate to the different biological activities, some of which have been developed as new drugs [3].

In the present study, polysaccharides were isolated from three seaweeds, namely one red algae (*Chondrus verrucosus*) and two brown alga (*Saccharina japonica* and *Undaria pinnatifida*), and their chemical characteristics and potential biological activities were compared.

Materials and methods

Chondrus verrucosus was collected on the coast of Kesennuma, Miyagi, Japan in May, 2016, and *S. japonica* and *U. pinnatifida* were collected on the coast of Onagawa, Miyagi, Japan in May, 2012. The three seaweeds were extracted three times with 0.17 N hydrochloric acid (final pH 2) at 65~70℃ for 1 hr. The dried material was dissolved in 250 ml of distilled water, and ethanol was added to the final concentration of 75% and crude polysaccharide was obtained [4]. It was further fractionated using anion-exchange chromatography [5,6].

Total sugar content of the polysaccharides was determined by the phenol-sulfuric acid method. The sulfate content was determined by the $BaCl_2$-gelatin turbidimetry method. The sugar product was treated with a trimethylsilylation reagent. The monosaccharide composition was analyzed by TMS-derivative with gas liquid chromatography (GLC).

Anti-hyaluronidase activity was assayed using modified Morgan-Elson method [7]. Cytotoxic levels of polysaccharides on RBL-2H3 cells were measured using the cell-counting kit-8 (CCK-8) assay. β-hexosaminidase inhibition activity was measured by A23187-stimulated assay [8]. A23187 (calcium Ionophore) (PubChem CID: 24277964) was purchased from Sigma-Aldrich. All the other reagents were of analytical grade.

Results

The total polysaccharides yielded from dried algae varied from 5.8% (*S. japonica*), 4.6% (*U. pinnatifida*) to 45.2% (*C. verrucosus*), indicating that *C. verrucosus* was richer in polysaccharides than the other two brown seaweeds (Table 1). The red algae showed higher sugar content in the ranges of 47.4%~76.8% than the two brown seaweeds (26.7%~40.1%), and higher sulfated content in the ranges of 12.5%~34.5% than two

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Table 1. The composition of polysaccharides in the three seaweeds

Species	Yield (% per dry weight)	Carbohydrate content (% per dry weight)	Sulfate content (% per dry weight)	Fucose (% per dry weight)
C. verrucosus	45.2	47.4~76.8	12.5~34.5	Not detected
S. japonica	5.8	26.7~40.1	7.8~10.7	2.0~18.9
U. pinnatifida	4.6	30.3	7	1.3

brown seaweeds (7%~30.3%). Fucose content of *S. japonica* fractions showed the highest value (18.9%), however, in the red algae it was not detected.

Polysaccharides inhibited hyaluronidase activity in a dose-dependent manner (data not shown). The activity of CV2 polysaccharides fraction was the strongest.

The 50% inhibitory concentration (IC_{50}) of the CV1 and the CV2 fraction from *C. verrucosus* were 0.110 mg/ml and 0.067 mg/ml, respectively. The IC_{50} of *S. japonica* and *U. pinnatifida* was 0.145 mg/ml and 2.35 mg/ml, respectively. It means the red algae showed the higher hyaluronidase inhibitory activity than the brown seaweeds.

Table 2. Effects of the polysaccharides on degranulation of RBL-2H3 cells (100 μg/ml)

Species	β-hexosaminidase inhibition activity (%)
C. verrucosus	73.2
S. japonica	63.1
U. pinnatifida	55.4

The three seaweeds inhibited the degranulation of RBL-2H3 cells stimulated by A23187 in a dose-dependent manner without cytotoxicity (data not shown). Polysaccharides affected the degranulation inhibition activity in a dose-dependent manner (data not shown). The higher anti-allergic activities might relate with the sulfate content (Table 2).

Discussion

Chemical composition of polysaccharides differed depending on the species. It means chemical composition of polysaccharides differed depending on the species. Some of the variations can be attributed to differences environmental condition.

According to the hyaluronidase inhibitory activity and β-hexosaminidase inhibitory activity results, polysaccharides fractions might function as an anti-allergic substance, but the mechanism is not yet clear.

Polysaccharides from three seaweeds of the higher anti-allergic activity might be related with their sulfate contents. However, further research is necessary to determine the mechanisms of the anti-allergic activity.

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