

Original article

The anti-allergic activities of polysaccharides from *Chondrus verrucosus* (Rhodophyta, Gigartinales)

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Introduction

Chondrus verrucosus is an important economic alga, and some of the local people in Japan consume for cooking. In the previous study, chitinase from *C. verrucosus* was reported to have strong chitinase activity [1].

To the best of our knowledge, there have been no studies about polysaccharides isolated from *C. verrucosus*. In this study, the anti-allergic activities of the *C. verrucosus* polysaccharides were examined to find out whether the polysaccharides fractions from *C. verrucosus* have the potential for treatment of inflammation.

Materials and methods

Chondrus verrucosus was collected on the coast of Kesennuma, Miyagi, Japan in May, 2016, and then extracted with each 3 volumes of 0.17 N hydrochloric acid (final pH 2) at 65~70°C for 1hr, three times. The dried material was dissolved in 250 ml of distilled water, finally, ethanol was added and crude polysaccharide was obtained [2], and fractionated by ion-exchange column chromatography [3,4].

Total sugar content of the polysaccharides was determined by the phenol-sulfuric acid method. The sulfate content was determined by the BaCl₂-gelatin turbidimetry method. The sugar product was treated with a trimethylsilylation reagent (TMS-PZ; Tokyo Kasei. Kogyo, Tokyo). The monosaccharide composition was analyzed by TMS-derivative with gas liquid chromatography (GLC). Galactose, xylose and glucose were used as standards.

Anti-hyaluronidase activity was assayed using modified Morgan-Elson method [5]. Cytotoxic levels of polysaccharides on RBL-2H3 cells were measured using the CCK-8 assay. β -hexosaminidase inhibition activity was measured by A23187-stimulated assay [6]. A23187 (calcium Ionophore) (PubChem CID: 24277964) was purchased from Sigma-Aldrich. All the other reagents were of analytical grade.

Results

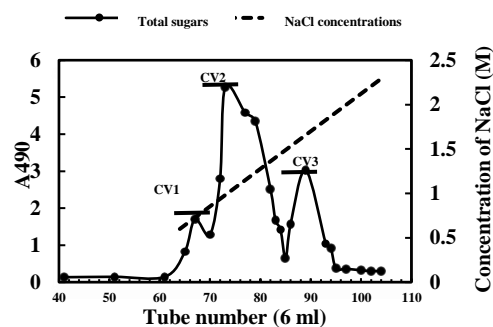


Fig. 1. Elution curve of *C. verrucosus* polysaccharides from Toyopearl DEAE-650 column chromatography. (CV1) Eluted with 0.69 M NaCl. (CV2) Eluted with 0.96 M NaCl. (CV3) Eluted with 1.69 M NaCl.

Polysaccharides were chromatographed on a Toyopearl DEAE-650 anion-exchange column resulting in three peaks, CV1, CV2 and CV3. The recoveries of CV1, CV2 and CV3 were 39.2%, 29.8% and 10.4%, respectively (Fig. 1).

The carbohydrate contents of CV1, CV2 and CV3 were 76.8%, 61.9% and 47.4%, respectively. It was found that galactose (86.1%, 78.3% and 45.2%, respectively) was the major sugar in the

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polysaccharides (Table 1).

Table 1. The chemical and monosaccharide compositions of polysaccharide fractions by ion exchange chromatography

Fractions of polysaccharides	Carbon hydrate content (%)	Sulfate content (%)	Monosaccharide composition (%)		
			Gal ^a	Glc ^a	Xyl ^a
CV1	76.8	34.5	86.5	2.1	3.2
CV2	61.9	42.0	78.3	5.8	5.5
CV3	47.4	12.5	45.2	7.2	9.4

^a Gal: galactose; Xyl: xylose; Glc: glucose

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

The 50% inhibitory concentration (IC₅₀) of CV1 and CV2 were 0.110 mg/ml and 0.067 mg/ml, respectively. The IC₅₀ of positive control (PC) used as the anti-allergic medicine was 0.174 mg/ml. It was revealed that CV1 and CV2 fractions had the similar anti-hyaluronidase activity to that of PC. These results suggested that the polysaccharide fractions might function as an anti-allergic substance.

Chondrus verrucosus polysaccharide fractions inhibited the degranulation of RBL-2H3 cells stimulated by A23187 in a dose-dependent manner without cytotoxicity (data not shown).

Discussion

The recoveries of polysaccharides fractions from *C. verrucosus* were very variable due to several factors, such as extraction methods, environmental conditions, seasonal variation and physiological factors.

In this study, the sulfated polysaccharide was extracted at higher temperature, in the higher sugar and sulfate content were obtained in the fractions, the result is consistent with the previous study (galactose content was 95.19%) [7].

According to the hyaluronidase inhibitory activity and β -hexosaminidase inhibition activity results, *C. verrucosus* polysaccharide fractions inhibited the degranulation of RBL-2H3 cells stimulated by A23187 in a dose-dependent manner without cytotoxicity. Presumably, the fractions may suppress the influx of Ca²⁺ into cells by chelating extracellular Ca²⁺. On the other hand, the fractions might be incorporated into cells and inhibit activation of intracellular signaling molecules, such as polysaccharide kinases.

From these results, polysaccharides fractions might function as an anti-allergic substance, but the mechanism have not yet been clear. Further research is necessary to determine the mechanisms of the anti-allergic activity.

Acknowledgments

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