

Available at www.sciencedirect.com

ScienceDirect

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

Studies on anti-allergic activity of *Sargassum horneri* extract

Hisashi Yoshioka^a, Momoko Ishida^b, Kosuke Nishi^b, Hiroshi Oda^a,
Haruhiko Toyohara^c, Takuya Sugahara^{b,d,*}

^a Maruha Nichiro Holdings, Inc., Tsukuba, Ibaraki 300-4295, Japan

^b Faculty of Agriculture, Ehime University, Matsuyama, Ehime 790-8566, Japan

^c Division of Applied Biosciences, Graduate School of Agriculture, Kyoto University, Kyoto, Kyoto 606-8502, Japan

^d Food and Health Science Research Center, Faculty of Agriculture, Ehime University, Matsuyama, Ehime 790-8566, Japan

ARTICLE INFO

Article history:

Received 16 January 2014

Received in revised form 27 May 2014

Accepted 2 June 2014

Available online

Keywords:

Anti-allergic activity

Sargassum horneri

Chlorophyll c2

Degranulation

Syk phosphorylation

RBL-2H3 cells

ABSTRACT

Chlorophyll c2 in a *Sargassum horneri* extract suppressed the degranulation of rat basophilic leukaemia RBL-2H3 cells. In this contribution, the mode of action of *S. horneri* extract was revealed. The extract inhibited the elevation of intracellular Ca^{2+} concentration induced by the antigen. Immunoblot analysis showed that *S. horneri* extract down-regulated the phosphorylation of both Syk and PI3K in the signalling pathways involved in degranulation triggered by antigen–antibody interaction. In addition, oral administration of *S. horneri* extract inhibited mice nasal rubbing behaviour sensitized with anti-DNP IgE. These findings suggest that *S. horneri* extract has anti-allergic activity via the suppression of degranulation of mast cells and basophils.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Many kinds of seaweeds have been consumed as foodstuff in Japan, Korea and parts of Europe. Seaweeds are rich in many bioactive compounds which can contribute to human health (Kumar & Brown, 2013; Rajapakse & Kim, 2011) such as polysaccharides, polyphenols, carotenoids, vitamins (Skrovankova, 2011), minerals (Misurcova, Machu, & Orsavova, 2011), and ω -3 and ω -6 polyunsaturated fatty acids (Misurcova, Amabrozova, & Samek, 2011; Miyashita & Hosokawa, 2010). Fucoidan and

carrageenan in the extracellular matrix of brown algae, have various biological functions, for example antiviral, anti-inflammatory, anticoagulant, anti-angiogenic, and anti-adhesive activities (Cumashi, Ushalova, & Preobrazhenskaya, 2007; Damonte, Matulewicz, & Cerezo, 2004; Prajapati, Maheriya, Jani, & Solanki, 2014). In addition, phlorotannins, a type of tannin in brown algae, are also reported to have an inhibitory effect on adipogenesis through AMP-activated protein kinase (AMPK) activation (Ko, Lee, Lee, Lim, & Jeon, 2013).

Sargassum horneri in the family *Sargassaceae* is an annual brown alga. *S. horneri* is distributed along the sea coast of Japan

* Corresponding author. Tel.: +81 89 946 9863; fax: +81 89 946 9863.

E-mail address: mars95@agr.ehime-u.ac.jp (T. Sugahara).

<http://dx.doi.org/10.1016/j.jff.2014.06.002>

1756-4646/© 2014 Elsevier Ltd. All rights reserved.

except for Hokkaido, and it has been eaten in Japan, especially in the Tohoku district, for a long time. It is reported that compounds in *S. horneri* have functional effects; for example, a protective effect of the chromene compound sargachromenol against UV-A induced skin damage (Kim, Ahn, Kong, & Kim, 2012), anticoagulant activity of high molecular weight polysaccharide or proteoglycan (Athukorala, Lee, Kim, & Jeon, 2007), and antioxidant activity of phenolics and fucoxanthin (Airanthi, Hosokawa, & Miyashita, 2011). Previously, we reported that an 80% aqueous ethanol *S. horneri* extract had an inhibitory effect on degranulation of a rat basophilic leukaemia cell line (RBL-2H3), and that the active substance was chlorophyll c2 (Chl-c2), one of the major pigments of brown algae (Yoshioka et al., 2013). RBL-2H3 cells are mucosal mast cells with functions similar to primary mast cells and normal basophils. Therefore, the assay using RBL-2H3 cells is a pertinent model for the study of allergic inflammation (Yamaki et al., 2007). Mast cell activation mediated by a high-affinity receptor for IgE (FcεRI) is considered a key event in the allergic inflammatory response. Kraft et al. (2007) reviewed the understanding of signalling pathways of degranulation after antigen–antibody interaction on mast cells.

After cross-linkage of IgE bound to its receptor FcεRI on mast cells, the primary pathway activates tyrosine-protein kinase Lyn, which phosphorylates β and γ immunoreceptor tyrosine-based activation motifs (ITAMs) in the FcεRI. Spleen tyrosine kinase (Syk) is then bound to the γ ITAMs and phosphorylated. The activated Syk phosphorylates linker for activation of T cells (LAT), which recruits phospholipase Cγ (PLCγ). PLCγ is phosphorylated through Syk and Bruton's tyrosine kinase (Btk). Phosphorylated PLCγ activates classical protein kinases C and induces Ca²⁺ mobilization. Protein kinase C activation and Ca²⁺ mobilization ultimately lead to degranulation by a calcium-dependent signalling pathway. The complementary pathway, which involves activation of Fyn and Syk by binding of IgE to its receptor, induces a signalling complex with growth-factor-receptor-bound protein 2 (GRB2) and GRB2-associated binding protein 2 (GAB2). GAB2 and Syk activate phosphoinositide 3-kinase (PI3K), and finally, activation of PI3K leads to degranulation by a calcium-independent signalling pathway. In this study, we investigated the mechanism of the suppressive effect of *S. horneri* extract on degranulation of RBL-2H3 cells stimulated with antigen. In addition, we evaluated the anti-allergic effect of *S. horneri* extract using an allergic rhinitis mouse model *in vivo*.

2. Materials and methods

2.1. Reagents

Dulbecco's Modified Eagle Medium (DMEM), penicillin, bovine serum albumin (BSA), mouse anti-dinitrophenol (DNP) monoclonal IgE, DNP-human serum albumin (HSA), cell lysis buffer, and Triton X-100 were obtained from Sigma (St. Louis, MO, USA). Aprotinin, Pefabloc SC, and protease inhibitor cocktail were obtained from Roche Applied Science (Basel, Switzerland). A phosphatase inhibitor cocktail was purchased from Nacalai Tesque (Kyoto, Japan). Goat anti-actin antibody and horseradish peroxidase (HSP)-labelled anti-goat IgG antibody were obtained

from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-PI3K antibody, anti-phosphorylated PI3K antibody, a B cell signalling antibody sampler kit including antibodies against Syk, phosphorylated Syk, Lyn, phosphorylated Lyn, Btk, phosphorylated Btk, a PLCγ antibody sampler kit including antibodies against PLCγ1, phosphorylated PLCγ1, PLCγ2, phosphorylated PLCγ2, and HRP-labelled anti-rabbit IgG antibody were purchased from Cell Signaling Technology (Danvers, MA, USA).

2.2. Sample preparation

The brown alga *S. horneri* was harvested from Matsushima Bay, Miyagi, Japan and Yamada Bay, Iwate, Japan. *S. horneri* 80% aqueous ethanol extract was prepared by our previous report (Yoshioka et al., 2013). Briefly, *S. horneri* was soaked in fresh water three times to remove salts, then dried at 25 °C for 12 h. Dried samples were powdered with a Foss sample mill (Hillerød, Denmark) and extracted in 80% aqueous ethanol for 15 h at 25 °C. After extraction, the solutions were filtered through filter paper (Toyo Roshi Kaisha, Ltd., Tokyo, Japan) and dried by evaporation. The dried extracts were stored at –80 °C until use.

2.3. β-Hexosaminidase release assay

Antigen-induced degranulation of basophilic cells was determined using a β-hexosaminidase release assay (Horigome et al., 2008). RBL-2H3 cells were obtained from the American Type Culture Collection (Rockville, MD, USA). The cells were cultured in DMEM supplemented with 10% FBS, 100 units/mL penicillin and 100 µg/mL streptomycin at 37 °C under humidified 5% CO₂–95% air.

RBL-2H3 cells were inoculated in a 24-well culture plate at 2.5×10^5 cells/well and cultured for 12 h at 37 °C. The cells were washed with phosphate-buffered saline (PBS), and sensitized with 25 ng/well of anti-DNP IgE for 2 h. After sensitization, to remove free anti-DNP IgE, the cells were washed twice with modified Tyrode's buffer (MT buffer) composed of 137 mM NaCl, 2.7 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂, 5.6 mM glucose, 20 mM HEPES, and 0.1% BSA. Next, MT buffer containing *S. horneri* 80% ethanol extract was added to each well and incubated for 10 min. Then 100 ng/well DNP-HSA was added and incubated for 30 min. The culture plate was then placed on ice to terminate antigen stimulation for 10 min, and the culture supernatant was collected from each well. Cell lysis buffer was then added, and the cells were disrupted by sonication to prepare cell lysate.

The culture supernatant or cell lysate was mixed with 0.1 M citrate buffer (pH 4.5) containing 3.3 mM *p*-nitrophenyl-2-acetoamide-β-D-glucopyranoside and the mixture was stored at 37 °C for 25 min. The absorbance at 405 nm of each reaction mixture was measured after terminating the enzymatic reaction by the addition of 2 M glycine buffer (pH 10.4). β-Hexosaminidase release was calculated using the following formula:

$$\beta\text{-hexosaminidase release (\%)} = \frac{(S - SB)}{(S - SB) + (C - CB)} \times 100$$

Where S is the absorbance of the culture supernatant, SB is the absorbance of the blank used for the culture superna-

tant, C is the absorbance of the cell lysate, and CB is the absorbance of the blank used for the cell lysate.

2.4. Measurement of intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$)

$[\text{Ca}^{2+}]_i$ was measured using a Calcium Kit-Fluo 3 (Dojindo Laboratories, Kumamoto, Japan) according to the manufacturer's instructions. RBL-2H3 cells were seeded into a white 96-well culture plate and treated with anti-DNP IgE as described above. Following IgE-sensitization, the cells were washed with PBS twice and incubated with 100 μL Fluo-3 AM for 1 h. Treated cells were washed again with PBS and incubated with *S. horneri* extract (200 $\mu\text{g}/\text{mL}$) or 80% aqueous ethanol as a control at 37 °C for 10 min. The cells were stimulated by the addition of 10 μL DNP-HSA diluted in MT buffer at 0.625 $\mu\text{g}/\text{mL}$, and the fluorescent intensity was immediately monitored at an excitation wavelength of 490 nm and an emission wavelength of 530 nm using an SH-8000Lab microplate reader (Corona Electric, Ibaraki, Japan).

2.5. Immunoblotting

Immunoblot analysis was performed as previously described (Ishida, Nishi, Watanabe, & Sugahara, 2013). RBL-2H3 cells were seeded into a 24-well culture plate at 2.5×10^5 cells/well and treated with anti-DNP IgE as described above. After washing the IgE-sensitized cells twice with MT buffer, 490 μL MT buffer containing *S. horneri* extract (200 $\mu\text{g}/\text{mL}$) or 80% aqueous ethanol as a control was added to each well and incubated at 37 °C for 10 min. The cells were then stimulated by addition of 10 μL DNP-HSA diluted in MT buffer at 2.5 $\mu\text{g}/\text{mL}$ and incubated for another 10 min at 37 °C. After removing the reagents, 30 μL lysis buffer containing 50 mM Tris, 150 mM NaCl, 1 mM ethylenediaminetetraacetic acid, 50 mM NaF, 30 mM $\text{Na}_4\text{P}_2\text{O}_7$, 2 $\mu\text{g}/\text{mL}$ aprotinin, the protease inhibitor cocktail, and the phosphatase inhibitor cocktail were added to each well. The cell lysates were centrifuged at $14,000 \times g$ at 4 °C for 15 min, and each supernatant was equalized to the same protein concentration. Proteins in the total cell lysate were then separated by SDS-PAGE and transferred onto a Hybond-P PVDF membrane (GE Healthcare, Little Chalfont, UK). The membrane was blocked with 5% skim milk dissolved in Tris-buffered saline containing 0.1% Tween 20 (TBS-T) for 1 h at room temperature. After washing with TBS-T, the membrane was incubated with primary antibody in 5% BSA-TBS-T at 4 °C overnight. The membrane was washed with TBS-T to remove excess primary antibody, and the membrane was incubated with HRP-labelled anti-goat IgG antibody or HRP-labelled anti-rabbit IgG antibody in 5% skim milk-TBS-T at room temperature for 1 h. After washing with TBS-T to remove excess HRP-conjugated antibody, blots were developed with ECL Western Blotting Detection Reagent (GE Healthcare) or ImmunoStar LD reagent (Wako Pure Chemical Industries). Bands were visualized using a ChemiDoc XPS Plus apparatus (Bio-Rad Laboratories, Inc. Hercules, CA, USA), and the chemiluminescent intensity was quantified using Quantity One software (Bio-Rad Laboratories).

2.6. Animal treatment

Four-week-old male C57BL/6 mice were purchased from Japan SLC, Inc. (Shizuoka, Japan). They were housed at two or four per cage and fed a basal diet (CRF-1; Oriental Yeast, Tokyo, Japan) and given water *ad libitum* at 19.7–23.1 °C and 29–64% humidity under a 12 h light/12 h dark cycle. After a week of acclimation, the mice were randomly divided into five groups (eight mice in each group).

Non-antigen stimulated group, the negative control group and the positive control group were orally administered 0.5% carboxymethylcellulose sodium (CMC) aqueous solution daily for 31 days (the experimental period). The *S. horneri* extract group was also administered *S. horneri* extract in 80% aqueous ethanol suspended in 0.5% CMC solution (0.2 g/kg or 1 g/kg body weight) daily for the experimental period. Body weight and mass of food intake were measured once a week. After 26 days, non-antigen stimulated group, the negative control group, the positive control group and the *S. horneri* extract group were injected via tail vein with 10 μg of anti-DNP IgE. After 5 days of IgE sensitization, the positive control group was administered olopatadine suspended in 0.5% CMC solution (0.003 g/kg body weight) and the mice, except for non-antigen stimulated group, were administered 50 μg of DNP-HSA by nasal drop; the incidence of nasal rubbing behaviour was then counted for 1 h. The study was performed in accord with the utilization manual for experimental animals of Maruha Nichiro Holdings, Inc. (Ibaraki, Japan).

2.7. Statistics

All results are expressed as means $\pm$ standard deviation (SD). Statistical differences among groups were evaluated by one-way analysis of variance (ANOVA) with Dunnett's test or student t test. Statistical analyses were conducted using StatLight #4 software (Yukms, Tokyo, Japan). Values of $p < 0.05$, $p < 0.01$ or $p < 0.001$ were considered to be statistically significant.

3. Results

3.1. Effect of *S. horneri* extract on degranulation of RBL-2H3 cells

S. horneri extract was added to culture medium of anti-DNP IgE-sensitized RBL-2H3 cells at various concentrations, and degranulation was induced by stimulation with DNP-HSA. As shown in Fig. 1, *S. horneri* extract inhibited the degranulation of RBL-2H3 cells stimulated by antigen in a dose-dependent manner. A cell viability test showed that *S. horneri* extract inhibited the degranulation of RBL-2H3 cells stimulated by antigen without cytotoxicity (data not shown). These observations indicated that *S. horneri* extract inhibits the degranulation of RBL-2H3 cells, whether involving the antigen-antibody interaction pathway.

3.2. Effect of *S. horneri* extract on $[\text{Ca}^{2+}]_i$ in RBL-2H3 cells

The elevation of intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) is the critical process in degranulation. Thus, the effect of *S. horneri*

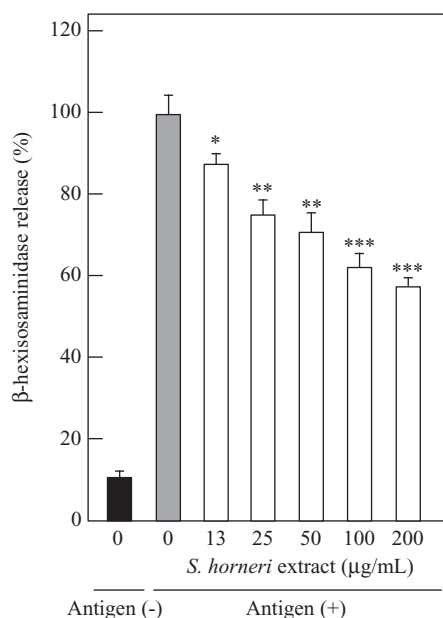


Fig. 1 – Inhibitory effect of *S. horneri* extract on the degranulation of RBL-2H3 cells stimulated by antigen. RBL-2H3 cells sensitized with anti DNP-IgE were incubated with various concentrations of *S. horneri* extract in 80% aqueous ethanol. After IgE sensitization, the cells were stimulated by DNP-HSA. The data are shown as β -hexosaminidase activity relative to control, and results are presented as the means $\pm$ SD ($n = 4$). Statistically significant differences compared with the control, analysed by Dunnett's test, are represented as * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

extract on $[Ca^{2+}]_i$ in RBL-2H3 cells was examined using Fluo-3 AM. As shown in Fig. 2, $[Ca^{2+}]_i$ increased and was sustained for 15 min after stimulation with antigen. In the presence of *S. horneri* extract, the elevation of $[Ca^{2+}]_i$ was suppressed at 5 min after stimulation and low Ca^{2+} level was sustained for 15 min.

3.3. Effect of *S. horneri* extract on the signalling pathways involved in antigen-stimulated degranulation

The mode of action of the suppressive effect of *S. horneri* extract on antigen-stimulated degranulation was revealed by immunoblotting. As shown in Fig. 3A, C, the phosphorylation levels of Syk, Btk and PLC γ 1/2 located downstream of the tyrosine kinase Lyn were suppressed by the addition of *S. horneri* extract, although the extract did not affect the phosphorylation of Lyn.

In addition, the phosphorylation level of PI3K was decreased by treatment with *S. horneri* extract (Fig. 3B, C). These results revealed that *S. horneri* extract suppresses antigen-induced degranulation in RBL-2H3 cells through down-regulation of both the Ca^{2+} -dependent signalling pathway and a Ca^{2+} -independent pathway involved in degranulation.

3.4. Effect of *S. horneri* extract on IgE-mediated nasal rubbing behaviour in mice

As shown in Fig. 4, the antigen stimulated group of mice (control) showed significant increase in nasal rubbing behaviour

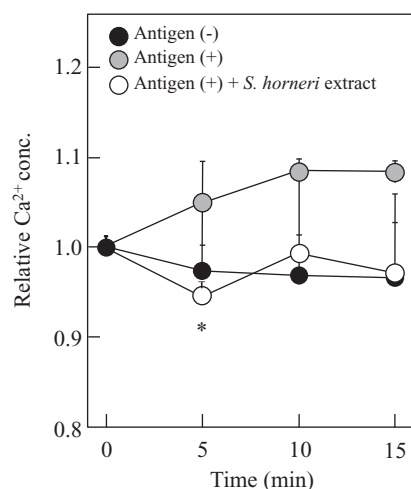


Fig. 2 – Effect of *S. horneri* extract on $[Ca^{2+}]_i$ in RBL-2H3 cells stimulated by antigen. $[Ca^{2+}]_i$ was measured by the Calcium Kit-Fluo-3. Anti-DNP IgE-sensitized RBL-2H3 cells were incubated with Fluo-3 AM for 1 h, then with *S. horneri* extract in 80% aqueous ethanol or 80% aqueous ethanol for 10 min. The treated cells were stimulated by DNP-HSA and fluorescence intensity was measured. (●) RBL-2H3 cells stimulated with antigen; (○) RBL-2H3 cells treated with 200 μ g/mL of *S. horneri* extract and stimulated with antigen; (●) unstimulated intact RBL-2H3 cells.

compared with the non-antigen stimulated group (intact group). The group administered olopatadine (positive control) showed significant suppression of nasal rubbing behaviour compared with the control group. When 0.2 or 1.0 g/kg body weight of *S. horneri* extract was orally administrated to IgE-mediated mice, both levels of extract significantly suppressed nasal rubbing behaviour.

4. Discussion

In our previous study, we reported that an *S. horneri* extract in 80% aqueous ethanol suppressed the antigen-induced degranulation of RBL-2H3 cells, and the active substance was identified as Chl-c2, one of the major pigments in brown algae (Yoshioka et al., 2013). However, the mode of action of *S. horneri* extract in having a suppressive effect on degranulation was unclear. Therefore, in this study, the mechanism underlying the inhibition by *S. horneri* extract was investigated. As shown in Fig. 1, *S. horneri* extract inhibited the degranulation of RBL-2H3 cells following an antigen-stimulated reaction. Ca^{2+} is one of the major second messengers in intracellular signalling, and the elevation of $[Ca^{2+}]_i$ is the critical process in degranulation. Thus, the effects of the extract on intracellular calcium levels were examined.

S. horneri extract suppressed the elevation of $[Ca^{2+}]_i$ in RBL-2H3 cells induced by antigen. These results implied that *S. horneri* extract suppresses the elevation of $[Ca^{2+}]_i$ following stimulation with antigen, thereby inhibiting degranulation on RBL-2H3 cells. To clarify how *S. horneri* extract affects the

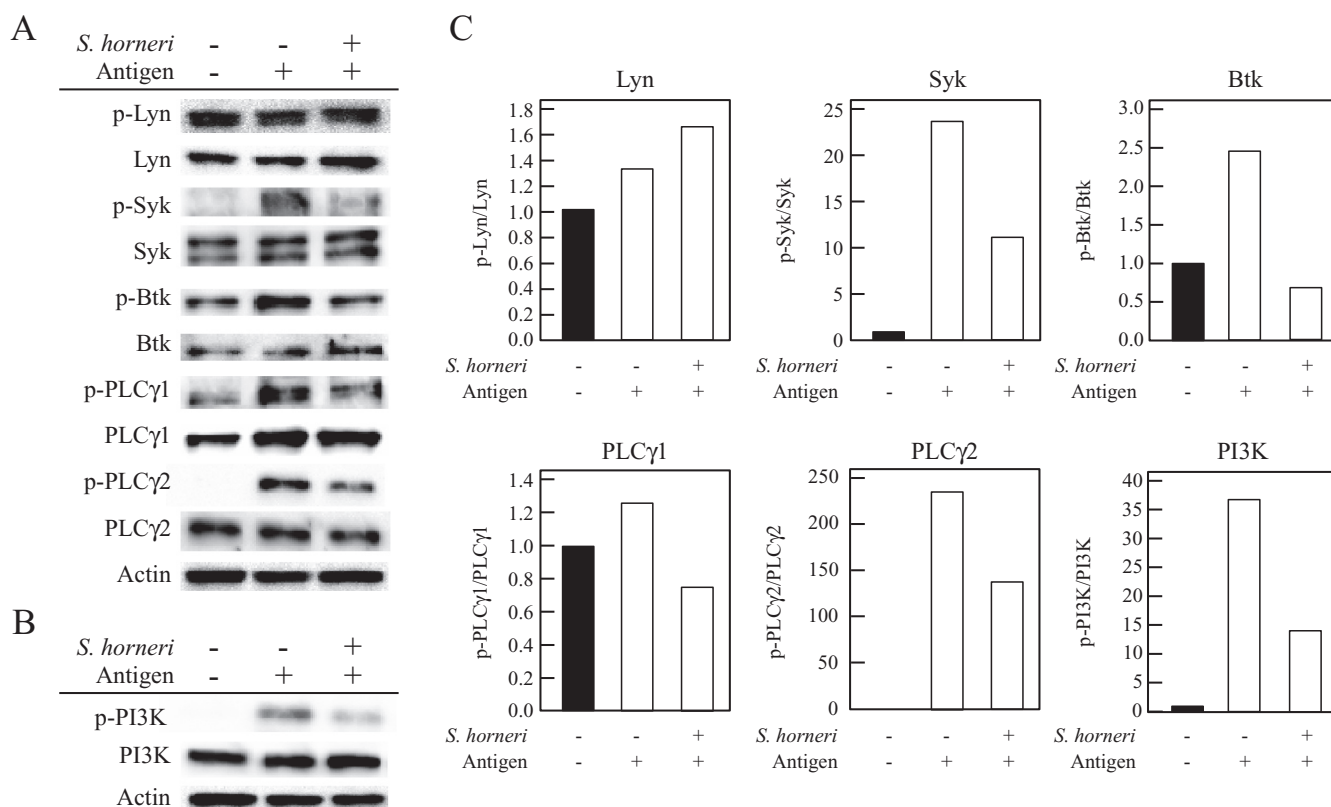


Fig. 3 – Effect of *S. horneri* extract on signalling pathways involved in degranulation of RBL-2H3 cells stimulated by antigen. (A): *S. horneri* extract was added to anti-DNP-IgE sensitized RBL-2H3 cells, and degranulation was induced by the addition of DNP-HSA. Each cell lysate was analysed by immunoblotting. The p-Lyn, p-Btk, p-Syk and p-PLC represent phosphorylated Lyn, phosphorylated Btk, phosphorylated Syk, and phosphorylated PLC, respectively. A representative blot from two independent experiments is shown. (B): *S. horneri* extract was added to anti-DNP-IgE sensitized RBL-2H3 cells, and degranulation was induced by the addition of DNP-HSA. Each cell lysate was analysed by immunoblotting. The p-PI3K represents phosphorylated PI3K. A representative blot from two independent experiments is shown. (C): The ratio of the p-Lyn/Lyn, p-Btk/Btk, p-Syk/Syk, p-PLC/PLC, and p-PI3K/PI3K in each cell lysate relative to that of intact cells (Antigen (–), *S. horneri* (–)).

signalling pathways involved in antigen-stimulated degranulation, we analysed immunoblots.

As seen in Fig. 3, *S. horneri* extract suppressed the phosphorylation levels of Syk, Btk, and PLCγ1/2, although it did not affect the phosphorylation level of Lyn, suggesting a distinct involvement of *S. horneri* extract in the Lyn–Syk–LAT signalling pathway. The results were consistent with the suppressed elevation of $[Ca^{2+}]_i$. In addition, *S. horneri* extract also suppressed the phosphorylation level of PI3K, suggesting that it might affect the Fyn–GAB2–PI3K pathway, which is a calcium-independent pathway.

The mode of action of purified Chl-c2, one of the active substances in *S. horneri*, was investigated. Chl-c2 suppressed the phosphorylation levels of Syk, Btk, and PLCγ1/2, but did not suppress phosphorylation of PI3K (data not shown). Sakai, Komura, Nishimura, Sugawara, and Hirata (2011) reported that phycoerythrin and phycoerythrobilin inhibit the calcium ionophore-induced degranulation of RBL-2H3 cells. Phycoerythrobilin also suppresses antigen-dependent degranulation but phycoerythrin does not. Phycoerythrobilin is the major light-harvesting pigment in red algae, while phycoerythrin is a water-soluble

chromoprotein that binds to phycoerythrobilin through cross-linking of its cysteine residues. Thus, the conformation of Chl-c2 in *S. horneri* extract might be different from purified Chl-c2, for example by combining with accessory pigments such as carotenoids.

Hong et al. (2011) reported that parthenolide, a sesquiterpene lactone isolated from the herb feverfew, inhibits degranulation and the elevation of $[Ca^{2+}]_i$ induced by antigen on RBL-2H3 cells. Parthenolide shows the same suppressive effects induced by calcium ionophore A23187. It was known that Ca^{2+} ionophore A23187 increase intracellular Ca^{2+} levels without antigen–antibody interaction, and induced degranulation of mast cells. Thus, A23187 was often used for studies on the mechanism of the suppressive effects of the active substances on degranulation. In addition, Hong et al. (2011) also reported that parthenolide suppressed the elevation of $[Ca^{2+}]_i$ levels stimulated by A23187. *S. horneri* extract also suppressed A23187-induced degranulation, and elevation of $[Ca^{2+}]_i$ levels (data not shown). Thus, *S. horneri* extract might implicate degranulation and the elevation of $[Ca^{2+}]_i$ levels stimulated by A23187. Further study is necessary to clarify the effect by which

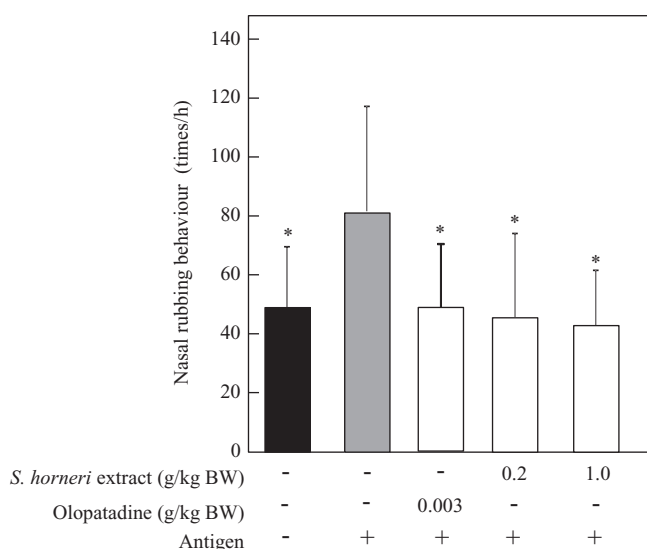


Fig. 4 – Suppression of IgE-mediated nasal rubbing behaviour in mice by *S. horneri* extract. After a week of acclimation, mice in non-antigen stimulated group (antigen (-), Olopatadine (-), *S. horneri* extract (-)), the negative control group (antigen (+), Olopatadine (-), *S. horneri* extract (-)), the positive control group (antigen (+), Olopatadine 0.003 g/kg BW, *S. horneri* extract (-)), *S. horneri* extract group (antigen (+), Olopatadine (-), *S. horneri* extract 0.2 or 1.0 g/kg BW) were orally administered daily for the experimental period. Twenty-six days after daily oral administration, those groups were injected with anti-DNP IgE in the tail vein. After 5 days of IgE sensitization, antigen was administered by nasal drop, except for non-antigen stimulated group, and then the incidence of nasal rubbing behaviour was counted for 1 h. Results are expressed as means $\pm$ SD ($n = 8$). Asterisks indicate statistically significant differences ($p < 0.05$) compared with the negative control group based on Student's *t* test.

S. horneri extract inhibits degranulation stimulated by a calcium ionophore.

In addition, the effect of orally administering *S. horneri* extract on antigen-stimulated C57BL/6 mice was examined. Oral administration of *S. horneri* extract significantly inhibited nasal rubbing behaviour. Sakai reported that fucoxanthin, a major pigment and carotenoid of brown algae, also suppressed the degranulation of mast cells and the mode of action of fucoxanthin was assumed to inhibit the translocation of FcεRI to lipid rafts (Sakai, Sugawara, Matsubara, & Hirata, 2009). Thus, *S. horneri* contains at least two substances, Chl-c2 and fucoxanthin, which contribute to the suppression of degranulation by different mechanisms of action.

This inhibitory effect was almost as strong as that of an anti-allergic drug, olopatadine (Fig. 4). These results indicated that administration of *S. horneri* extract may alleviate IgE-mediated allergic reactions *in vivo*. In conclusion, our results demonstrated that *S. horneri* extract suppresses degranulation of mast cells stimulated by antigen. This effect was shown to result from both inhibition of Ca^{2+} mobilization through down-regulated

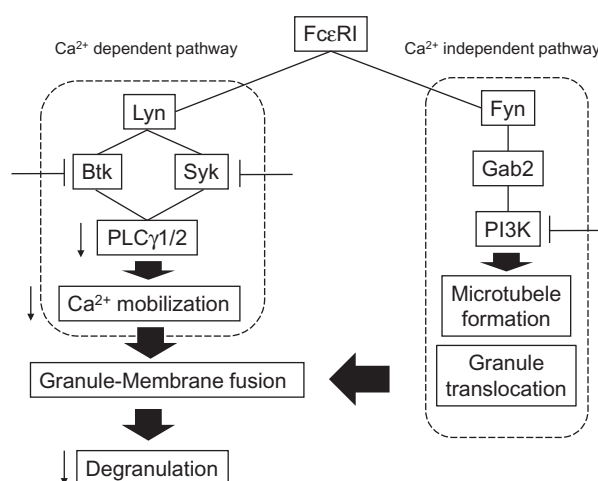


Fig. 5 – The mechanism of inhibition by *S. horneri* extract on degranulation of RBL-2H3 cells stimulated by antigen. *S. horneri* extract in 80% aqueous ethanol inhibited the phosphorylation of Syk and Btk. It caused down-regulation of the phosphorylation of PLCγ1/2 and Ca^{2+} mobilization, ultimately suppressing degranulation on mast cells. *S. horneri* extract also inhibited the phosphorylation of PI3K and suppressed degranulation through the Ca^{2+} -independent pathway.

phosphorylation of the Lyn–Syk–LAT signalling pathway and suppression of the Fyn–GAB2–PI3K pathway (Fig. 5).

In addition, orally administering *S. horneri* extract to mice inhibited an IgE-mediated allergic reaction *in vivo*. Thus, *S. horneri* 80% ethanol extract may be useful for the prevention or mitigation of allergic symptoms.

Acknowledgements

The authors would like to thank Mr. A. Kamata, Mr. T. Konishi, Mr. J. Takahashi and Mr. M. Shimada for their valuable comments, Mr. E. Shimanoki for his technical assistance. We continue to hope and pray for the recovery of the Tohoku district, especially Miyagi and Iwate, Japan's major kelp-producing coasts for *Sargassum horneri*, from the 2011 Tohoku earthquake and tsunami.

REFERENCES

- Airanthi, M. K., Hosokawa, M., & Miyashita, K. (2011). Comparative antioxidant activity of edible Japanese brown seaweeds. *Journal of Food Science*, 76, 104–111.
- Athukorala, Y., Lee, K.-W., Kim, S.-K., & Jeon, Y.-J. (2007). Anticogulant activity of marine green and brown algae collected from Jeju Island in Korea. *Bioresource Technology*, 98, 1711–1716.
- Cumashi, A., Ushalova, M. A., & Preobrazhenskaya, M. E. (2007). A comparative study of anti-inflammatory, anticoagulant, antiangiogenic, and antiadhesive activities of nine

- different fucoidans from brown seaweeds. *Glycobiology*, 17, 541–552.
- Damonte, E. B., Matulewicz, M. C., & Cerezo, A. S. (2004). Sulfated seaweed polysaccharides as antiviral agents. *Current Medicinal Chemistry*, 11, 2399–2419.
- Hong, J., Aoyama, S., Hirasawa, N., Zee, O., Ishihara, K., Hashida, C., Kimura, M., Seyama, T., & Ohuchi, K. (2011). Suppression of intracellular calcium levels and inhibition of degranulation in RBL-2H3 mast cells by the sesquiterpene lactone parthenolide. *Planta Medica*, 77, 252–256.
- Horigome, S., Yoshida, I., Tamaki, C., Yamaguchi, A., Kibune, N., Kamide, T., & Watai, M. (2008). Simple screening method for inhibitory effects of food ingredients on degranulation in RBL-2H3 cells. *Nippon Shokuhin Kagaku Kogaku Kaishi*, 55, 535–540.
- Ishida, M., Nishi, K., Watanabe, H., & Sugahara, T. (2013). Inhibitory effect of aqueous spinach extract on degranulation of RBL-2H3 cells. *Food Chemistry*, 136, 322–327.
- Kim, J.-A., Ahn, B.-N., Kong, C.-S., & Kim, S.-K. (2012). Protective effect of chromene isolated from *Sargassum horneri* against UV-A-induced damage in skin dermal fibroblasts. *Experimental Dermatology*, 21, 630–642.
- Ko, S. C., Lee, M., Lee, J. H., Lim, Y., & Jeon, Y. J. (2013). Dieckol, a phlorotannin isolated from a brown seaweed, *Ecklonia cava*, inhibits adipogenesis through AMP-activated protein kinase (AMPK) activation in 3T3-L1 preadipocytes. *Environmental Toxicology and Pharmacology*, 36, 1253–1260.
- Kraft, S., & Kinet, J. P. (2007). New developments in FcεRI regulation, function and inhibition. *Nature Reviews Immunology*, 7, 365–378.
- Kumar, S. A., & Brown, L. (2013). Seaweeds as potential therapeutic interventions for the metabolic syndrome. *Reviews in Endocrine and Metabolic Disorders*, 14, 299–308.
- Misurcova, L., Amabrozova, J., & Samek, D. (2011). Seaweed lipids as nutraceuticals. *Advances in Food and Nutrition Research*, 64, 339–355.
- Misurcova, L., Machu, L., & Orsavova, J. (2011). Seaweed minerals as nutraceuticals. *Advances in Food and Nutrition Research*, 64, 371–390.
- Miyashita, K., & Hosokawa, M. (2010). Future direction of lipid nutrition and development of functional foods – Gift from seaweeds and its application. *Journal of Lipid Nutrition*, 19, 39–45.
- Prajapati, V. D., Maheriya, P. M., Jani, G. K., & Solanki, H. K. (2014). Carrageenan: A natural seaweed polysaccharide and its applications. *Carbohydrate Polymers*, 105, 97–112.
- Rajapakse, N., & Kim, S.-K. (2011). Nutritional and digestive health benefits of seaweed. *Advances in Food and Nutrition Research*, 64, 17–28.
- Sakai, S., Komura, Y., Nishimura, Y., Sugawara, T., & Hirata, T. (2011). Inhibition of mast cell degranulation by phycoerythrin and its pigment moiety phycoerythrobilin, prepared from *Porphyr a yezoensis*. *Food Science and Technology Research*, 17, 171–177.
- Sakai, S., Sugawara, T., Matsubara, K., & Hirata, T. (2009). Inhibitory effect of carotenoids on degranulation of mast cells via suppression of antigen-induced aggregation of high affinity IgE receptors. *The Journal of Biological Chemistry*, 284, 28172–28179.
- Skrovankova, S. (2011). Seaweed vitamins as nutraceuticals. *Advances in Food and Nutrition Research*, 64, 357–369.
- Yamaki, K., Taneda, S., Yanagisawa, R., Inoue, K., Takano, H., & Yoshino, S. (2007). Enhancement of allergic responses in vivo and in vitro by butylated hydroxytoluene. *Toxicology and Applied Pharmacology*, 223, 164–172.
- Yoshioka, H., Kamata, A., Konishi, T., Takahashi, J., Oda, H., Tamai, T., Toyohara, H., & Sugahara, T. (2013). Inhibitory effect of chlorophyll c2 from brown algae, *Sargassum horneri*, on degranulation of RBL-2H3 cells. *Journal of Functional Foods*, 5, 204–210.