Leukotrienes-Mediated Effects of Water Extracts from *Sargassum horneri*, a Marine Brown Alga, on Cl⁻ Absorption in Isolated Rat Colon

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Abstract: *Sargassum horneri* is an edible marine brown alga distributed along the seacoast of Japan. Here we examined effects on the water-soluble (ethanol-insoluble) extracts (EIS) from *Sargassum horneri* on ion transports across the isolated rat colonic mucosa set in Ussing chambers. The nonpolysaccharide fraction of EIS (EIS-2) significantly decreased short-circuit current (I_{sc}) across the mucosa, and increased the tissue conductance (G_{t}). The half-maximal effect of EIS-2 was obtained at 20 μg/ml. In contrast, the polysaccharide fraction of EIS (EIS-1; 100 μg/ml) had little effect on I_{sc} and G_{t}. The effect of EIS-2 depended on the presence of Cl⁻ and HCO₃⁻ but not K⁺ in the bathing solution. These results suggest that EIS-2 stimulates Cl⁻ absorption in the colonic mucosa. The EIS-2-induced changes in I_{sc} and G_{t} were inhibited by 3-((1-[p-chlorobenzyl]-5-[isopropyl]-3-t-butylthiophen-2-yl)-2,2-dimethyl-propanoic acid sodium (MK-886; 10 μM), a 5-lipoxygenase-activating protein inhibitor, and 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB; 100 μM), a Cl⁻ channel blocker. EIS-2 attenuated the prostaglandin E₂ (0.5 μM)-increased I_{sc}, and the half-maximal effect of EIS-2 was obtained at 50 μg/ml. The present study suggests that the EIS-2 stimulates Cl⁻ absorption mediated by basolateral leukotriene-sensitive Cl⁻ channels and apical Cl⁻/HCO₃⁻ exchanger in the rat colonic mucosa. [The Japanese Journal of Physiology 54: 71–77, 2004]

**Key words:** rat colon, marine alga, leukotriene, chloride absorption.

The effects of toxic extracts from marine algae on the activities of several ion transporting proteins are attractive and have been reported well; for example, caulerpenyne, a toxin from *Caulerpa taxifolia*, inhibits Na⁺,K⁺-ATPase in leech touch neurons [1], and the toxin from *Prymnesium patelliferum* activates voltage-dependent Ca²⁺ channels in rat anterior pituitary GH4Cl cells [2]. On the other hand, the effects of extracts obtained from nontoxic marine algae on ion transport are generally less attractive and have not been studied well.

Edible marine algae have been highlighted recently as multifunctional foods for maintaining human health. They are rich in minerals, vitamins, and dietary fibers. *Sargassum horneri* is one of these edible brown marine alga and is distributed along the seacoast of Japan [3]. Slightly boiled alga has been used as a savory food in Japan [4]. It is interesting that water extracts of *Sargassum horneri* have a stimulatory effect on bone formation in rat *in vitro* [5] and show antiviral activity against herpes simplex virus type I, human cytomegalovirus, and human immuno-deficiency virus type I [4, 6].

In the present study, using isolated rat colonic mucosa we tested if the water extracts from *Sargassum horneri* affect K⁺ and Cl⁻ transports and suggest that they stimulate Cl⁻ absorption across the colonic mucosa.
MATERIALS AND METHODS

Chemicals. Indomethacin and tetrodotoxin were obtained from Wako Pure Chemical Industries (Osaka, Japan), 3-(1-[p-chlorobenzyl]-5-[isopropyl]-3-t-butyliothioindol-2-yl)-2,2-dimethyl-propanoic acid sodium (MK-886) was from BIOMOL Research Laboratories (Plymouth Meeting, PA, USA), and 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB) was from Research Biochemicals International (Natick, MA, USA). Indomethacin was dissolved in ethanol, and NPPB and MK-886 were dissolved in dimethyl sulfoxide. Ethanol and dimethyl sulfoxide concentrations in the final solutions never exceeded 0.5%, at which concentration the vehicle per se did not affect the short-circuit current ($I_{sc}$), the potential difference across the mucosa ($P_d$), or the tissue conductance ($G_t$).

Water extracts from Sargassum horneri (EIS-1 and EIS-2). Sargassum horneri was collected on the seacoast near an island of Notojima in Ishikawa Prefecture, Japan, in April 2002. Fresh algal material (0.96 kg) was cut into pieces and extracted twice with 2 l of boiling water for 1 h. After concentration of the hot water extract, three volumes of ethanol were added to the residue. The resulting precipitate was filtered and washed with cold ethanol. The precipitate was dissolved in water, then lyophilized to give a brownish residue. This ethanol-insoluble extract is named EIS. The yield of EIS was 27.5 g. On the other hand, the ethanol-soluble supernatant of the hot water extracts was evaporated to dryness under reduced pressure. This fraction was dissolved in water, then lyophilized to give brown residue. This ethanol-soluble extract is named ES. The yield of ES was 41.2 g.

ES (1 g) was dissolved in 0.01 M citrate buffer, pH 7.0, containing 0.1 M NaCl, and the soluble portion was applied to a column of Sepharose 6B (4.4×93 cm; Pharmacia Biotech, Uppsala, Sweden). The column was eluted with the same buffer, and fractions of each 20 ml were collected. The eluted fractions were separated into two fractions (EIS-1 and EIS-2), according to the elution profile prepared on the basis of the phenol–H$_2$SO$_4$ method at 480 nm and UV absorbance at 260 nm [6]. The early eluted fraction (EIS-1) was polysaccharide-containing fraction because the phenol–H$_2$SO$_4$ reaction was positive. On the other hand, a fraction (EIS-2) eluted later showed strong UV absorption at 260 nm, but phenol–H$_2$SO$_4$ reaction was weak. Therefore EIS-2 might be a non-polysaccharide fraction. Both fractions were dialyzed against water (MWCO 14,000, Wako Pure Chemical Industries) and lyophilized. The yields of EIS-1 and EIS-2 were 0.31 and 0.39 g, respectively.

Tissue preparation. The following procedures were performed in accordance with the guidelines presented by the Animal Care and Use Committee of Toyama Medical and Pharmaceutical University. The mucosa-submucosa preparation (hereafter simply described as the mucosa) were obtained from female Sprague-Dawley rats (Japan SLC, Shizuoka, Japan) with a weight of 140–200 g [7, 8]. The animals had free access to water and food until the day of the experiment. They were killed rapidly by stunning and cervical dislocation. The serosa and muscularis propria were stripped away by hand to obtain the mucosa preparation of the distal part of the colon descendens. The Parsons solution (normal bathing solution) for tissue preparation and Ussing chamber experiments consisted of (in mM): 107 NaCl, 4.5 KCl, 25 NaHCO$_3$, 1.8 Na$_2$HPO$_4$, 0.2 NaH$_2$PO$_4$, 1.25 CaCl$_2$, 1 MgSO$_4$, and 12 glucose. The solution was gassed with 5% CO$_2$–95% O$_2$ at a pH of 7.4. For the K$^+$-free solution, KCl was replaced by NaCl. For the low Cl$^-$ solution, NaCl was replaced by sodium gluconate and supplemented with 4.5 mM CaSO$_4$ to compensate the Ca$^{2+}$-buffering properties of gluconate [9]. The HCO$_3$$^-$-free solution consisted of (in mM) 140 NaCl, 4.5 KCl, 1.25 CaCl$_2$, 1 MgSO$_4$, 10 HEPES, and 12 glucose. The solution was adjusted to pH 7.4 with NaOH and gassed with 100% O$_2$.

Ussing chamber experiments. The tissue was fixed in a modified Ussing chamber and bathed with 4 ml of the Parsons solution incubated at 37°C on each side of the mucosa [8, 10, 11]. The exposed surface of the tissue was 0.3 cm$^2$. A short-circuit current ($I_{sc}$) was continuously measured at zero voltage difference with an amplifier (CEZ-9100, Nihon Kohden Co., Tokyo, Japan). The fluid resistance was compensated. The direction of $I_{sc}$ from the mucosal to serosal side was expressed as positive: That is, an increase in Cl$^-$ movement from the mucosal to serosal side ($I_{sc}$) was measured at zero voltage difference with an amplifier (CEZ-9100, Nihon Kohden Co., Tokyo, Japan). The fluid resistance was compensated. The reference was taken on the mucosal side. Tissue conductance ($G_t$) was determined from the deviation of $I_{sc}$ in response to the command voltage pulse of 0.5 mV (its duration was 100 ms).

To check the leak of K$^+$ from salt bridges into the solution in the Ussing chamber, K$^+$ concentration of the modified K$^+$-free solution (130 mM choline chloride, 1.25 mM CaCl$_2$, 1 mM MgSO$_4$, and 10 mM HEPES, pH 7.4) in the chamber was monitored by using PBFI tetraammonium salt (Molecular Probes,
Eugene, OA, USA). The K⁺ concentration was slightly increased from 0 to 0.39±0.02 mM during a 2 h incubation at 37°C (n=3).

The osmolalities of the normal bathing solution, the solution containing EIS-1 (100 μg/ml), and the solution containing EIS-2 (100 μg/ml) were measured with a microosmometer (Advanced Instruments, Norwood, MA, USA) and determined to be 274±1, 271±1, and 271±1 mosmol/kg H₂O, respectively (n=3). The presence of EIS-1 (100 μg/ml) or EIS-2 (100 μg/ml) did not change the pH of the bathing solution (data not shown).

**Statistical analysis.** Results are presented as the means±SE. The differences between groups were analyzed by one-way analysis of variance (ANOVA), and correction for multiple comparisons was made by using Dunnett’s multiple comparison test. A comparison between the two groups was made by using the Student’s t-test. Statistically significant differences were assumed at p<0.05.

**RESULTS**

**Effects of water extracts from Sargassum horneri on electrical parameters in isolated rat colonic mucosa**

The ethanol-insoluble fraction from water extracts (EIS) of *Sargassum horneri* (100 μg/ml at the mucosal side) decreased basal $I_{sc}$ and increased $G_t$ and $P_d$ in isolated rat colonic mucosa set in Ussing chambers, but the ethanol-soluble fraction from water extracts (ES) of the alga (100 μg/ml at the mucosal side) had no significant effect on these parameters (data not shown). The EIS was subsequently separated into two fractions with a Sepharose 6B column. As shown in Fig. 1, EIS-2 (100 μg/ml at the mucosal side) significantly changed the $I_{sc}$ and $G_t$, but EIS-1 (100 μg/ml at the mucosal side) had little effects on these parameters. EIS-2 (100 μg/ml) also changed the $P_d$ from 2.8±0.2 to 2.3±0.2 mV ($p<0.05$, n=4). The decrease in $I_{sc}$ and the accompanying increase in $G_t$ (Fig. 1E, F) suggest that EIS-2 stimulates K⁺ secretion and/or Cl⁻ absorption in the colonic mucosa.

**Stimulation of Cl⁻ absorption by EIS-2**

A decrease in $I_{sc}$ (Fig. 2A) and an increase in $G_t$ (Fig. 2B) induced by EIS-2 (100 μg/ml at the mucosal side) were not dependent on K⁺ ions in the bathing solution. The half-maximal effect on $I_{sc}$ was obtained at 20 μg/ml (Fig. 2C). EIS-2 (100 μg/ml) significantly changed the $P_d$ from 2.8±0.3 to 1.7±0.2 mV in the K⁺-free solution ($p<0.01$, n=5), as found in the normal bathing solution.

![Fig. 1. Effects of EIS-1 and EIS-2 on electrical parameters in isolated rat colonic mucosa. A and D: Effects of EIS-1 (100 μg/ml at the mucosal side) (A) and EIS-2 (100 μg/ml at the mucosal side) (D) on the $I_{sc}$ in the normal bathing solution. Typical traces are shown. B and E: The values of $I_{sc}$ just before (open column) and 25 min after (filled column) the addition of EIS-1 (B) or EIS-2 (E) were read. The data are means±SE from three experiments. NS, not significantly different (p>0.05). * Significantly different (p<0.05). C and F: The values of $G_t$ just before (open column) and 25 min after (filled column) the addition of EIS-1 (C) or EIS-2 (F) were read. The data are means±SE from three experiments. NS, not significantly different (p>0.05). * Significantly different (p<0.05).](image)

On the other hand, the EIS-2 (100 μg/ml)–induced effects on these parameters were abolished in the low-Cl⁻ bathing solution containing 7 mM Cl⁻ (Fig. 3A–C). Moreover, NPPB (100 μM at the serosal side), a blocker of the Cl⁻ channel in the rat colon [12, 13], suppressed the EIS-2-induced effects. That is, in the presence of NPPB, EIS-2 (100 μg/ml) little changed the values of $I_{sc}$ and $G_t$ from 10.8±0.6 to 8.6±1.8 μA/cm² ($p>0.05$) and from 7.4±0.5 to 7.4±0.5 mS/cm² ($p>0.05$), respectively (n=4). These results suggest that the EIS-2-response is caused by an absorption of Cl⁻ ions and that the serosal Cl⁻ channels are involved in the Cl⁻ absorption.

The EIS-2–induced effects were dependent on the presence of HCO₃⁻ (Fig. 3D–F), suggesting that apical Cl⁻/HCO₃⁻ exchanger also contributes to the Cl⁻ absorption caused by EIS-2.
EIS-2–stimulated Cl\(^{-}\)/HCO\(_3\) absorption is mediated by leukotrienes

We checked whether arachidonic acid metabolites are involved in the regulation of the EIS-2-induced Cl\(^{-}\)/HCO\(_3\) absorption in the rat colon. The effect of EIS-2 on Cl\(^{-}\) absorption was abolished by the preincubation of the mucosa with MK-886 (10 \(\mu\)M at the serosal and the mucosal sides), a 5-lipoxygenase-activating protein inhibitor [14] (Fig. 4A–C), but the effect of EIS-2 was not inhibited by the preincubation with indomethacin (1 \(\mu\)M at the serosal and the mucosal sides), a cyclooxygenase inhibitor (Fig. 4D–F). The indomethacin-induced decrease in Isc (Fig. 4D) was probably due to the inhibition of a synthesis of endogenous cyclooxygenase metabolites, which stimulate Cl\(^{-}\) secretion in the colon [7, 8]. Indomethacin (1 \(\mu\)M) significantly decreased the value of \(G_t\) to 6.5\(\pm\)0.2, from 7.2\(\pm\)0.3 mS/cm\(^2\) (\(p<0.05\), \(n=3\)). These results suggest that the EIS-2–induced effect was mediated by leukotrienes, but not by prostaglandins or thromboxane. In the presence of tetrodotoxin (1 \(\mu\)M at the serosal side), EIS-2 (100 \(\mu\)g/ml) significantly changed the values of \(I_{sc}\) and \(G_t\) from 35.1\(\pm\)2.1 to 28.8\(\pm\)0.8 \(\mu\)A/cm\(^2\) (\(p<0.05\)) and from 6.3\(\pm\)0.2 to 7.2\(\pm\)0.2 mS/cm\(^2\) (\(p<0.05\)), respectively (\(n=4\)), suggesting that the EIS-2–induced effect was not mediated by a stimulation of secretomotor neurons.

Effect of EIS-2 on the prostaglandin E\(_2\)–induced Cl\(^{-}\) secretion

Prostaglandin E\(_2\) (0.5 \(\mu\)M at the serosal side) stimulates Cl\(^{-}\) secretion in the rat colonic mucosa (Fig. 5A, C), as reported previously [10, 15]. The addition of EIS-2 to the mucosal side inhibited the prostaglandin E\(_2\)–induced Cl\(^{-}\) secretion in a concentration-dependent manner, and the half-maximal inhibitory effect was observed at 50 \(\mu\)g/ml (Fig. 5B). The EIS-2 (100 \(\mu\)g/ml)–sensitive \(I_{sc}\) in the presence of prostaglandin E\(_2\) (32.7\(\pm\)1.9 \(\mu\)A/cm\(^2\), \(n=5\)) was much larger than that in the absence of prostaglandin E\(_2\) (7.9\(\pm\)1.6 \(\mu\)A/cm\(^2\), \(n=4\)). In the presence of prostaglandin E\(_2\) (0.5 \(\mu\)M), EIS-2 (100 \(\mu\)g/ml) signifi-
cantly increased the value of $G_t$ (Fig. 5C).

**DISCUSSION**

In the present study, we prepared two kinds of water-soluble (ethanol-insoluble) extracts (EIS) from *Sargassum horneri*, an edible marine alga. The EIS-1, a polysaccharide fraction of EIS, has been found to show potent antiviral activity against herpes simplex virus type I, human cytomegalovirus, and human immunodeficiency virus type I [4, 6]. Here, EIS-2, a nonpolysaccharide fraction of EIS, decreased $I_{sc}$ and increased $G_t$ in isolated rat colonic mucosa (Figs. 1–3). In the present experimental condition, a stimulation of $Cl^-$/$H^+$ absorption should accompany the decrease in $I_{sc}$ and the increase in $G_t$, and an inhibition of $Na^+$/H$^+$ absorption should accompany the decreases in $I_{sc}$ and $G_t$. Therefore EIS-2 may cause the $Cl^-$ absorption in the rat colonic mucosa. A direct measurement of $Cl^-$ flux is necessary to establish that the EIS-2–induced decrease in $I_{sc}$ is really due to $Cl^-$ absorption. In contrast, EIS-1 had no effect on the ion transport in isolated rat colonic mucosa (Fig. 1).

In rat colonic mucosa, several bioactive materials such as peptide YY [16], sorbin [17], butyrate [13], and leukotriene D$_4$ [18] have been reported to stimulate $Cl^-$ absorption. The $Cl^-$ absorption induced by peptide YY is mediated via the presynaptic and postsynaptic sites in the distal colon [16]. The butyrate-induced $Cl^-$ absorption accompanying the decrease in $I_{sc}$ is mediated by a release of leukotriene D$_4$, and two factors are responsible for this mechanism. (1) The in-
tracellular production of HCO$_3^-$ during the oxidation of butyrate as substrate for the apical Cl$^-$/HCO$_3^-$ exchanger, and (2) the activation of volume- and leukotriene D$_4$-sensitive basolateral Cl$^-$ channels [18]. NPPB inhibits the leukotriene D$_4$-sensitive Cl$^-$ channel in the rat colonic crypts.

It is interesting that the present effect of EIS-2 on the colonic electrical parameters was diminished by MK-886, a 5-lipoxygenase–activating protein inhibitor (Fig. 4A–C), and NPPB, the Cl$^-$ channel blocker. Moreover, the effect of EIS-2 depended on the presence of HCO$_3^-$ (Fig. 3D–F). Our results suggest that EIS-2 stimulates the Cl$^-$ absorption mediated by basolateral leukotriene-sensitive Cl$^-$ channels and the apical Cl$^-$/HCO$_3^-$ exchanger.

The effect of EIS-2 on the Cl$^-$ absorption was not inhibited by indomethacin (Fig. 4D–F), suggesting that the EIS-2–induced effect is not mediated by endogenous prostaglandins or thromboxane. It has been reported that exogenously administrated cyclooxygenase metabolites and their analogues—such as prostaglandin E$_2$ [19, 20], prostaglandin F$_{2a}$ [20], iloprost (a prostaglandin I$_2$ derivative) [19], and STA$_2$ (a stable thromboxane A$_2$ analogue) [8, 10, 21]—stimulated Cl$^-$ secretion in rat and rabbit colons. In the present study, we found that the effect of EIS-2 was still evident in the presence of prostaglandin E$_2$ (Fig. 5). Prostaglandin E$_2$ stimulates Cl$^-$ secretion via an activation of the cAMP-dependent apical Cl$^-$ channels [19]. Thus a stimulation of Cl$^-$ absorption by EIS-2 via the opening of the basolateral Cl$^-$ channel accompanied the $G_i$ increase even in the presence of prostaglandin E$_2$ (Fig. 5C).

Diarrhea is generally accompanied with an excess secretion of electrolytes, especially Cl$^-$ [22]. In the diarrheal disease, the increase in stool volume is the consequence of a situation in which the volumes presented to the colon exceed its large reserve capacity. A stimulation of water absorption in the colon represents an important target to reduce stool output in secretory diarrhea [17].

In conclusion, we suggest that the EIS-2 extracted from Sargassum horneri, a marine brown alga, stimulates Cl$^-$ absorption in isolated rat colonic mucosa. The stimulation of the absorption by EIS-2 is mediated by lipooxygenase metabolites. EIS-2 may be a good candidate for use in the reduction of water and electrolyte losses that occur in the diarrhea.

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