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Isolation of a New Phlorotannin, a Potent Inhibitor of Carbohydrate-Hydrolyzing Enzymes, from the Brown Alga *Sargassum patens*

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ABSTRACT: Ethanol extracts from 15 kinds of marine algae collected from the coast of the Noto Peninsula in Japan were examined for their inhibitory effects on human salivary α-amylase. Four extracts significantly suppressed the enzyme activity. An inhibitor was purified from the extract of *Sargassum patens*. The compound was a new phloroglucinol derivative, 2-(4-(3,5-dihydroxyphenoxy)-3,5-dihydroxyphenoxy) benzene-1,3,5-triol (DDBT), which strongly suppressed the hydrolysis of amylopectin by human salivary and pancreatic α-amylases. The 50% inhibitory activity (IC_{50}) for α-amylase inhibition of DDBT (3.2 μg/mL) was much lower than that of commercially available α-amylase inhibitors, acarbose (26.3 μg/mL), quercetagetin (764 μg/mL), and α-amylase inhibitor from *Triticum aestivum* (88.3 μg/mL). A kinetic study indicated that DDBT was a competitive α-amylase inhibitor with a K_i of 1.8 μg/mL. DDBT also inhibited rat intestinal α-glucosidase with an IC_{50} value of 25.4 μg/mL for sucrase activity and 114 μg/mL for maltase activity. These results suggest that DDBT, a potent inhibitor of carbohydrate-hydrolyzing enzymes, may be useful as a natural nutraceutical to prevent diabetes.

KEYWORDS: *Phloroglucinol derivative, α-amylase inhibitor, α-glucosidase inhibitor, Sargassum patens*

INTRODUCTION

Diabetes is one of the most serious chronic diseases associated with the increase in obesity and aging in the general population. The control of postprandial hyperglycemia is believed to be important in the treatment of diabetes and prevention of cardiovascular complications. One of the therapeutic approaches to this control is to retard glucose absorption by inhibiting carbohydrate-hydrolyzing enzymes, such as α-amylase and α-glucosidase, in the digestive organs. Acarbose from microorganisms is an inhibitor of both α-amylase and α-glucosidase used for lowering postprandial glucose levels in diabetic patients.[1−3] However, its continuous use may cause undesirable side effects, such as flatulence, abdominal cramping, vomiting, and diarrhea. For this reason, natural products that inhibit these enzymes with no side effects are sought.

Marine algae are an underexploited plant resource and a source of functional food. They are also rich sources of structurally diverse bioactive compounds with great pharmaceutical and biomedical potential, including pigments, fucoidans, phycocolloids, and phlorotannins. Phlorotannins, more commonly known as marine algal polyphenols, occur in the Phaeophyceae and are generally constructed of phloroglucinol (1,3,5-trihydroxybenzene) moieties linked by aryl−aryl bonds, aryl−ethyl bonds, or bonds of a mixed type. Phlorotannins display a variety of biological activities, including antioxidant,[4−7] anticoagulant,[8] antiallergic,[9] antihyperlipidemic,[10] algicidal,[11] and enzyme-inhibitory effects.[12−15]

In the present study, we focused on the isolation of a potential antidiabetic material with α-amylase inhibitory activity in marine algae. A total of 15 kinds of algae were collected from the coast of the Noto Peninsula to the north of the Sea of Japan, Ishikawa, Japan. Algae are plentiful around the peninsula, and many species can be collected from the coast.[16] We report here the isolation of a new phlorotannin from an alga, *Sargassum patens* (Japanese name Yatsumata-moku) and the *in vitro* evaluation of its inhibitory activities against α-amylase and α-glucosidase.

MATERIALS AND METHODS

Materials. Marine algae were collected from the coast of the Noto Peninsula in March 2009, January 2010, and March 2010. The samples were washed with tap water to remove salt and sand attached to the surface and stored in a medical refrigerator at −80 °C. α-Amylases from human pancreas and saliva, intestinal acetone powder from rat, and α-amylase inhibitor from *Triticum aestivum* (wheat) seeds were purchased from Sigma-Aldrich (St. Louis, MO). Acarbose was purchased from LKT Laboratories, Inc. (St. Paul, MN). Quercetagetin was purchased from Extrasynthese SA (Lyon Nord, France). Amylopectin and soluble starch were purchased from Nacalai Tesque (Kyoto, Japan). The protein concentrations of amylases were determined from their absorbance at 280 nm using the known values of $E^{1\%}$ = 25 cm^{-1} and molecular weight (MW) = 56 000. All other reagents were of analytical grade.

Preparation of Algal Extraction. Prior to extraction, the frozen algal samples were lyophilized, homogenized with a grinder, and passed through a sieve of 0.5 mm mesh. For the ethanol extract, algal powder (1 g) was added to ethanol (40 mL) and suspended with a

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homogenizer. The suspension was centrifuged after incubation with gentle shaking for 1 h at 25 °C. The supernatant was retained, and the precipitate was suspended in 40 mL of ethanol and incubated under the same conditions. This solution was centrifuged, and the supernatant obtained was mixed with the previously retained supernatant. The volume of the mixed solution was adjusted to 100 mL with ethanol.

Screening for the Inhibitory Activity of the Algal Extract against α-Amylase. The α-amylase inhibitory assay was performed using the iodo-starch reaction. To prepare the enzyme solution, 100 nM human salivary α-amylase was prepared with 20 mM Tris-HCl (pH 7.2). A 1% (w/v) solution of soluble starch in the same buffer was used as the substrate. For the assay, 35 μL of algal extract, 5 μL of substrate solution, and 10 μL of enzyme solution were mixed in a microtiter plate. After incubation for 10 min at room temperature, 150 μL of an iodine and potassium iodide solution [0.1% (w/v) I_2 and 1% (w/v) KI] was added and the absorbance was measured at 595 nm. The positive control (in which the algal extract and the enzyme solution were replaced by respective carrier solvents) and the negative control (in which the algal extract, the enzyme solution, and the substrate solution were replaced by respective carrier solvents) were assayed in the same way as described for the algal extract. The inhibitory activity (%) of the algal extract was calculated using the following equation:

$$\text{inhibitory activity of the algal extract} = [1 - (A - A^-)/(A^+ - A^-)] \times 100$$

where A, A^-, and A^+ were absorbances of the algal extract, negative control, and positive control, respectively. The extent of inhibition is expressed as the concentration necessary for 50% inhibitory activity (IC_{50}).

Isolation of Phlorotannin with α-Amylase Inhibitory Activity. The ethanol extract from *S. patens* was evaporated at 40 °C to dryness. The dry material was dissolved in 50% (v/v) methanol and separated with *n*-hexane, chloroform, and ethyl acetate. The ethyl acetate fraction exhibiting the desirable α-amylase inhibitory activities was evaporated at 40 °C, dissolved in 10% (v/v) acetonitrile, and filtered through an Ultrafree-LH filter (Merck Millipore, Darmstadt, Germany). Finally, the active compounds were purified by high-performance liquid chromatography (HPLC). The filtrate was loaded on an Inertsil Ph-3 column (4.6 × 250 mm; GL Science, Tokyo, Japan) in 10% (v/v) acetonitrile and eluted stepwise with acetonitrile in three concentrations: 10% (v/v) for 10 min, 50% (v/v) for 15 min, and 100% (v/v) for 15 min. The flow rate was 1 mL/min, and absorbance at 660 nm was monitored. Active fractions eluting between 15.5 and 17.6 min were pooled and lyophilized.

Effect of 2-(4-(3,5-Dihydroxyphenoxy)-3,5-dihydroxyphenoxy) Benzene-1,3,5-triol (DDBT) on Carbohydrate Hydrolysis of Human Salivary and Pancreatic α-Amylases. Human salivary and pancreatic α-amylases were dissolved at 1 μM concentration in 100 mM sodium phosphate buffer (pH 7.0). Amylopectin solution (1%, w/v) was prepared in the same buffer, and DDBT was dissolved in 10% (v/v) ethanol. Next, 10 μL of the enzyme solution was added to the reaction mixture containing 300 μL of the DDBT solution, 140 μL of the substrate solution, and 50 μL of 10 mM $CaCl_2$, followed by incubation at 37 °C. At 0, 2, 5, 10, 15, 20, 30, 40, 50, and 60 min after addition, the mixture was assayed according to the method by Walker and Harmon,[17] with some modifications: 50 μL of the mixture was withdrawn, and the reaction was stopped by the addition of 500 μL of 2% (w/v) sodium carbonate containing 10 mM potassium ferricyanide and boiling for 10 min. The absorbance of the reaction solution diluted with water was measured at 415 nm. The amount of liberated reducing sugars was quantified as maltose units using a calibration curve prepared with a reference standard. For the assay in the absence of inhibitors, the enzyme solution was replaced by carrier solvent and assayed in the same way as described for the DDBT solution. A maltose concentration of 8.2×10^{-3} M was expected to be produced under the assumption that amylopectin was digested completely under the experimental conditions used. Digestion of amylopectin (%) was expressed as (maltose concentration in the sample − maltose concentration in the sample control)/$(8.2 \times 10^{-3}) \times 100$.

α-Amylase Inhibitory Activities of DDBT and Commercially Available α-Amylase Inhibitors. The α-amylase inhibitory assay was performed using human salivary α-amylase. α-Amylase inhibitor from *T. aestivum* was dissolved in 100 mM sodium phosphate buffer (pH 7.0), and the other inhibitors were dissolved in 10% (v/v) ethanol. Enzyme solutions (1 μM, 10 μL) were added to reaction mixtures containing 300 μL of inhibitor solution, 140 μL of 1% (w/v) amylopectin solution, and 50 μL of 10 mM $CaCl_2$, followed by incubation at 37 °C. At 0 and 5 min after the addition, 50 μL of the mixture was withdrawn and the mixture was assayed according to the potassium ferricyanide method described above. For the positive control assay, the inhibitor solution was replaced with respective carrier solvents. The negative control (in which the inhibitor and enzyme solutions were replaced by respective carrier solvents) and the sample control (in which the enzyme solution was replaced by carrier solvent) were assayed in the same way as described above. Percentage inhibitory activity was expressed as [1 − (maltose concentration in the presence of the inhibitor − maltose concentration in the sample control)/(maltose concentration in the positive control − maltose concentration in the negative control)] × 100.

α-Glucosidase Inhibitory Activity of DDBT. Rat pancreatic acetone powder (0.5 g) was homogenized in 10 mL of 100 mM potassium phosphate buffer (pH 6.8) and 30 mL of distilled water. The supernatant after centrifugation was used as an α-glucosidase solution. DDBT was dissolved in 10% (v/v) ethanol. For the assay of maltase and sucrase activities, maltose and sucrose were used as respective substrates. The enzyme solution (25 μL) was added to the reaction mixture containing 25 μL of DDBT solution and 50 μL of 100 mM substrate solution, followed by incubation at 37 °C. At 30 min after the addition, the mixture was heated at 100 °C for 3 min to stop the reaction. After centrifugation, the glucose concentration in the supernatant was determined with Glucose CII Test Wako (Wako Pure Chemical Industries, Osaka, Japan) according to the protocol of the supplier. The assays in the absence of DDBT, negative control, and sample control were performed in the same way as described for α-amylase inhibitory assay, except for using rat pancreatic α-glucosidase as enzyme and maltose and sucrose as substrates. Percent inhibitory activity was expressed as [1 − (glucose concentration in the presence of the inhibitor − glucose concentration in the sample control)/(glucose concentration in the positive control − glucose concentration in the negative control)] × 100.

Kinetic Analysis. The reaction mixture consisted of different concentrations of amylopectin [0.07−1.12% (w/v)] as the substrate and 0.02 μM human salivary α-amylase in 100 mM sodium phosphate buffer (pH 7.0) containing 1 mM $CaCl_2$. The initial velocity v was obtained from the concentration of liberated reducing sugar in the reaction for 5 min at 37 °C, determined using the potassium ferricyanide method as described above. The Michaelis constant K_m and maximal velocity V_{max} of human salivary α-amylase were determined from Lineweaver−Burk plots. The inhibition constant K_i of the competitive inhibitor was calculated using the following equation: $1/v = K_m(1 + [\text{DDBT}]/K_i)/(V_{max}[\text{amylopectin}]) + 1/V_{max}$.

Structure of the Active Compound. The active compound was a brownish yellow powder. MS (m/z): 375.2 (MH^+), $C_{18}H_{14}O_9$, DDBT (calculated: 374.0638). Elemental analysis: C, 57.76; H, 3.77; O, 38.47. The infrared spectrum of the compound revealed the presence of a hydroxy group (3200 cm^{-1}) and an aromatic nucleus (1608 cm^{-1}) and showed an intrinsic phenol peak at 1263 cm^{-1}. ^1H NMR (500 MHz, CD_3OD) δ: 5.90 (1 H, bs), 5.92 (1 H, bs), 5.94 (1 H, bs), 6.00 (1 H, bs), 6.04 (1 H, bs), 6.15 (1 H, bs), 6.29 (1 H, bs). ^{13}C NMR (500 MHz, CD_3OD) δ: 95.8, 97.1 (×4), 98.8 (×2), 125.8, 126.8, 153.0 (×2), 153.2, 153.4, 154.8 (×2), 157.1 (×2), 158.9.

RESULTS

Screening for α-Amylase Inhibitory Activity of Marine Algae Extracts. To discover sources of inhibitory activity against carbohydrate-hydrolyzing enzymes, ethanol extracts

prepared from 15 kinds of marine algae were screened for their inhibitory activity against human salivary α-amylase (Table 1).

Table 1. Residual Activity of α-Amylase in the Presence of Algal Extracts and IC_{50} Values of α-Amylase Inhibition

species	residual activity (%)	IC_{50} (mg/mL)
brown algae		
Coccophora langsdorfii	98.3	
Sargassum ringgoldianum subsp. *coreanum*	22.5	0.03
Sargassum micracanthum	99.0	
Sargassum siliquastrum	6.96	0.02
Sargassum thunbergii	98.1	
Sargassum patens	14.4	0.05
Sargassum piluliferum	19.2	0.06
Petalonia binghamiar	91.0	
Sphaerotrichia divaricata	94.5	
Ecklonia kurome	97.8	
Sargassum fulvellum	98.6	
Undaria pinnatifida	95.3	
Ecklonia cava	96.5	
Nemacystus decipiens	96.8	
green alga		
Enteromorpha	99.1	

Obvious inhibitory activity was observed in four extracts, from *Sargassum ringgoldianum* subsp. *coreanum*, *Sargassum siliquastrum*, *S. patens*, and *Sargassum piluliferum*. IC_{50} values ranged from 0.02 to 0.06 mg of dry algal weight/mL (Table 1), suggesting that the algae contained compounds with α-amylase inhibitory activity. Of the four algae, only *S. patens* (Japanese name Yatsumata-moku) has been reported to be edible. Therefore, we focused on *S. patens* as a source of α-amylase inhibitor for potential antidiabetic use and evaluated its inhibitory activity.

Isolation of α-Amylase Inhibitor from *S. patens*. The procedure for isolation of the α-amylase inhibitor component is shown in Figure 1. The ethanol extract from freeze-dried powder of *S. patens* was partitioned with *n*-hexane, chloroform, and ethyl acetate. Because the ethyl acetate fraction showed the maximum α-amylase inhibitory activity, the active compounds were purified from the ethyl acetate fraction by HPLC using a phenyl column. As shown in Figure 2, the eluent was collected into seven fractions, Ph1−Ph7, and assayed for α-amylase inhibitory activity. The Ph6 fraction, eluted between 15.5 and 17.6 min, showed inhibitory activity, while the other fractions did not.

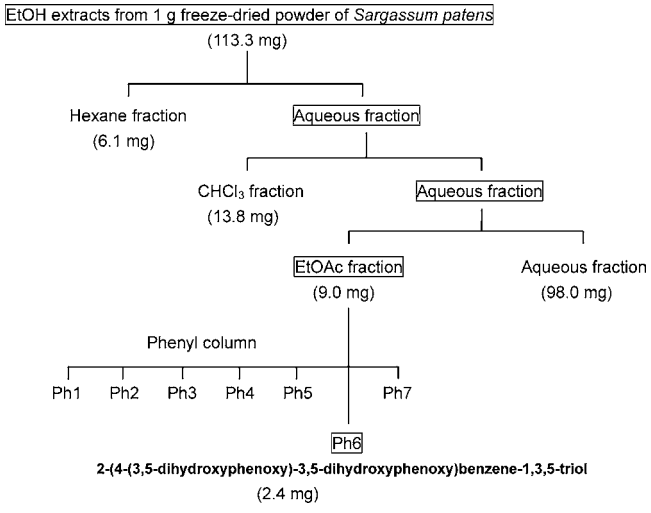

Figure 1. Schematic diagram of the procedure for isolation of an α-amylase inhibitor, DDBT, from *S. patens*. Boxed fractions showed α-amylase inhibitory activity. Figures in parentheses represent dry weights of the respective fractions.

A compound obtained from the fraction showed the following characteristic signals as phlorotannin. From the ^{1}H NMR spectrum, only the broad signal in which two or more signals have overlapped with 5.90−6.29 ppm was observed. This belonged with the aromatic protons, which have an oxygen substitution at both *ortho* positions. From the ^{13}CNMR spectrum, signals that overlapped at 153.0−158.9, 125.8−126.8, and 95.8−98.8 ppm, were observed. The integration intensity ratio of these three signals was 9:2:7. We assigned these three signals as the aromatic carbons of the root of an oxygen substitution, 2 position of carbons of 1,2,3-oxygen-substituted benzene structure, and 2 position of carbons of 1,2-oxygen-substituted benzene structure, respectively. The compound exhibited a positive-ion peak at m/z 375.2 in the mass spectrum. On the basis of these results, we concluded the compound as a new phloroglucinol derivative, DDBT (Figure 3). Content of DDBT in the alga was calculated as 0.7 mg/wet gram of alga.

Effect of DDBT on Carbohydrate Hydrolysis of Human Salivary and Pancreatic α-Amylases. The effect of DDBT on carbohydrate hydrolysis of human salivary and pancreatic α-amylases was investigated using amylopectin as a substrate. Figure 4 shows time courses of amylopectin digestion in the presence and absence of DDBT. In the absence of DDBT, amylopectin was progressively digested over time and the digestion yields for 60 min were 62.9% for salivary and 49.9% for pancreatic α-amylase, whereas the respective digestion yields in the presence of DDBT decreased to 15.4 and 2.9%. These results clearly show that DDBT inhibited both salivary and pancreatic α-amylases.

Comparison of Inhibitory Activity of DDBT with Commercially Available α-Amylase Inhibitors. The inhibitory activity of DDBT was compared to those of the commercially available α-amylase inhibitors acarbose and quercetagetin and an α-amylase inhibitor from *T. aestivum*. Acarbose is an oligosaccharide obtained from fermentation of a microorganism, *Actinoplane* sp., and is known to be an effective inhibitor of both α-amylase and α-glucosidase.[1−3] Quercetagetin is a flavonoid that has been suggested to interact with the active site of α-amylase, resulting in its inhibition.[18] α-Amylase inhibitor from *T. aestivum* is a salivary-type α-amylase inhibitor.[19−21] Table 2 shows IC_{50} values of DDBT, acarbose, quercetagetin, and the *T. aestivum* α-amylase inhibitor for inhibition of α-amylase activity. The value for DDBT is much smaller than that of the other inhibitors. The IC_{50} values were calculated as 8.6 μM for DDBT, 41 μM for acarbose, 2.4 mM for quercetagetin, and 4.2 μM for *T. aestivum* α-amylase inhibitor from the result given in Table 2 using their MW values of 374, 645.6, 318.25, and 21 000, respectively. These values indicate that DDBT is an efficient inhibitor of α-amylase.

Inhibitory Effect of DDBT on α-Glucosidase. In our ongoing effort to obtain a natural inhibitor of carbohydrate-hydrolyzing enzymes, we also evaluated the inhibitory activity of DDBT on α-glucosidase. DDBT inhibited both maltase and

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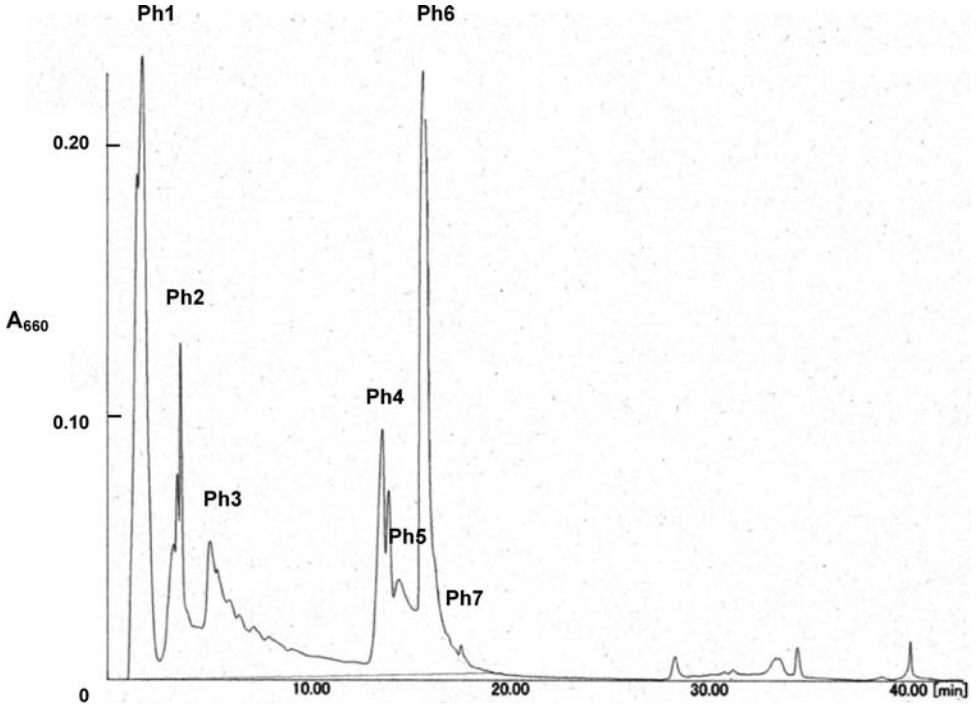

Figure 2. HPLC chromatogram of the ethyl acetate fraction of ethanol extraction from *S. patens*. The fraction was loaded onto an Inertsil Ph-3 column. Elution was performed with a solvent system of CH_3CN/H_2O (10:90 for 10 min, 50:50 for 15 min, and 100:0 for 15 min) and a flow rate of 1.0 mL/min at room temperature. Peak fractions Ph1−Ph7 were manually collected under absorbance monitoring at 660 nm.

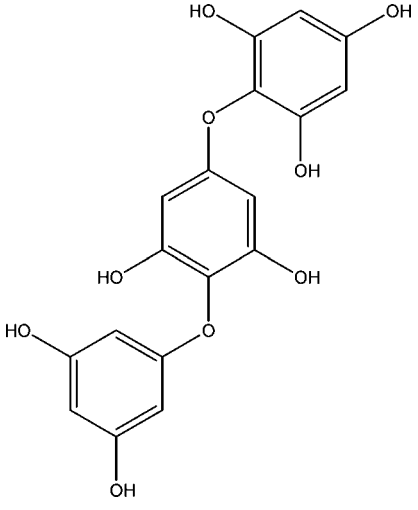

Figure 3. Chemical structure of DDBT.

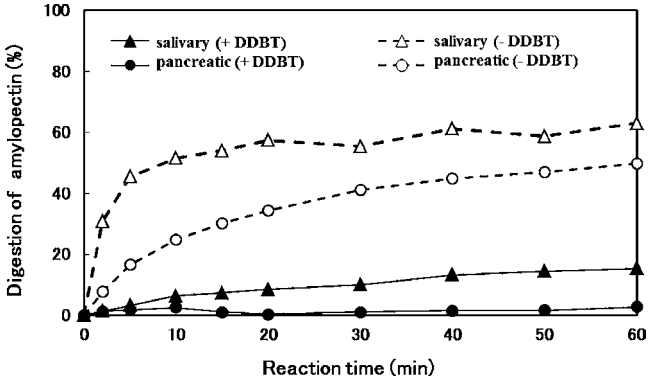

Figure 4. Time courses of amylopectin digestion by human salivary and pancreatic α-amylases in 100 mM sodium phosphate buffer (pH 7.0) containing 1 mM $CaCl_2$ at 37 °C. The reaction was carried out in the absence or presence of 10 μg/mL DDBT. The enzyme concentration was 0.02 μM. The data points present the average of two experiments.

sucrase activities of α-glucosidase (Table 3), but it was more effective in inhibiting α-amylase than α-glucosidase (Table 3).

Kinetics of Inhibitory Activity of DDBT against α-Amylase. A kinetic study of the inhibitory effect of DDBT on human salivary α-amylase was performed using amylopectin as the substrate. The initial velocity v of the enzyme was measured at various substrate concentrations $[S]$ (Figure 5). In the absence of DDBT, K_m and V_{max} were calculated as 0.27% and 4.8×10^{-4} M/min, respectively, from the double-reciprocal plots of the data (inset of Figure 5). As shown in Figure 5, DDBT lowered v and the double-reciprocal plots of the data gave a straight line with the y intercept at almost the same position as in the absence of DDBT (K_m = 0.58%, and V_{max} = 4.5×10^{-4} M/min; inset of Figure 5). This result showed that DDBT was a competitive inhibitor with a K_i of 1.8 μg/mL.

DISCUSSION

Phlorotannins, a class of polyhydroxylated phenols occurring in the Phaeophyceae, have been reported to exhibit various biological activities, e.g., radical-scavenging activity,[4,22] anti-allergic activity,[9] tyrosinase inhibition activity,[13] antihyperlipi-

Table 2. α-Amylase Inhibitory Activity of DDBT and Other Inhibitors

substances	IC_{50} (μg/mL)
DDBT	3.2
acarbose	26.3
quercetagetin	764
α-amylase inhibitor from wheat	88.3

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Table 3. α-Glucosidase Inhibitory Activity of DDBT

enzyme activity	IC_{50} (μg/mL)
α-glucosidase maltose activity	114
α-glucosidase sucrose activity	25.4

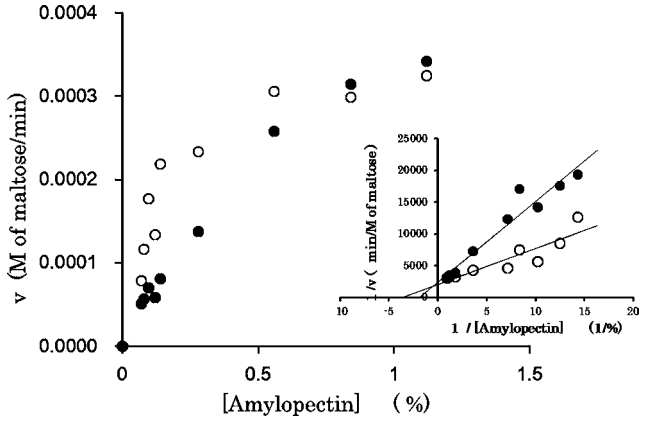

Figure 5. Kinetic study of human salivary α-amylase inhibition by DDBT in 100 mM sodium phosphate buffer (pH 7.0) containing 1 mM $CaCl_2$ at 37 °C. The reaction was carried out in the absence (○) or presence (●) of 2 μg/mL DDBT. The enzyme concentration was 0.02 μM. (Inset) Lineweaver−Burk plots for inhibition. The data points present the average of two experiments.

demic effect,[10] anti-inflammatory activity,[5] inhibitory effect on human immunodeficiency virus type I reverse transcriptase,[14] antiplasmin inhibitory activity,[8] antiplasmin inhibitory activity,[23] and algicidal activity on red-tide microalgae.[11] Although inhibitory activities of phlorotannins against α-amylase are relatively rare, some reports have been published: diphlorethohydroxycarmalol isolated from *Ishige okamurae*[12] showed an IC_{50} value of 0.53 mM, while 1-(3′,5′-dihydroxyphenoxy)-7-(2″,4″,6″-trihydroxyphenoxy)-2,4,9-trihydroxydibenzo-1,4-dioxin, eckol, and dieckol obtained from *Eisenia bicyclis* showed respective percent inhibition at 1 mM of 89.5, 87.5, and 97.5%.[15] The IC_{50} value of DDBT for α-amylase inhibition was 8.6 μM as described above. Thus, DDBT was found to be a much more effective α-amylase inhibitor than the reported phlorotannins.

Polyphenolic compounds are known to associate with a variety of proteins to form complexes. Piparo et al. investigated the association of 19 kinds of flavonoids, which are polyphenolic compounds of terrestrial plants, with human salivary α-amylase.[18] They measured the α-amylase inhibitory activity of the flavonoids while computationally modeling the interaction between the flavonoids and the active site of the enzyme using a ligand docking program. On the basis of the relationship between the inhibitory activity and the interaction, they proposed that the activity depended upon the formation of hydrogen bonds between the OH groups of the flavonoids and the carboxylate groups of Asp197 and Glu233 in the active site of human salivary α-amylase. Although the conformation of DDBT has not been elucidated, we speculate that DDBT can assume a conformation similar to that of a flavonoid upon binding with human salivary α-amylase. In this conformation, the OH groups of DDBT may play the same role in the association with the side chains of the active site as proposed for the OH groups of flavonoids, resulting in inhibition of enzyme activity.

In the process of carbohydrate digestion in mammals, α-amylase and α-glucosidase rapidly convert digestible starch into glucose monomers, which are absorbed by intestinal villus cells. Inhibition of the carbohydrate-hydrolyzing enzymes delays the digestion of starch and is effective in preventing an excessive postprandial rise in the blood glucose level. In this study, we assayed the inhibition activity of algal extracts against carbohydrate-hydrolyzing enzymes represented by human salivary α-amylase. The phlorotannin isolated from *S. patens* DDBT was a competitive inhibitor of human salivary α-amylase and showed marked inhibitory activity against human pancreatic α-amylase (Figure 4). Humans have five α-amylase genes, three encoding salivary α-amylase and two encoding pancreatic α-amylase. Both salivary and pancreatic α-amylases are composed of a single polypeptide chain of 496 amino acids. They share a high degree of amino acid sequence similarity, with 97% identical residues overall and 92% in the catalytic domains.[24−27] This similarity strongly suggests that DDBT interacts with both enzymes by a similar catalysis-inhibitory mechanism. DDBT also inhibited α-glucosidase (Table 3). These results suggest that DDBT is a potent antihyperglycemic agent that may be valuable as an inhibitor of carbohydrate-hydrolyzing enzymes.

DDBT was isolated from *S. patens*, a species of brown algae inhabiting the coast around the Noto Peninsula in Ishikawa Prefecture of Japan. This alga grows in close association of an edible brown alga, *Nemacystus decipiens* Kuckuck (Japanese name Ito-mozuku), that is widely consumed as Japanese salad, Sunomono. After the harvest of *N. decipiens* by separation from *S. patens*, residual *S. patens* drifts ashore to the coast of the Noto Peninsula and is discarded as waste. We showed in this study that *S. patens* contains a new phlorotannin, DDBT, with a pronounced inhibitory effect on carbohydrate-hydrolyzing enzymes. Because DDBT may delay the absorption of dietary carbohydrates in the digestive organs, leading to suppression of an increased blood glucose level after a meal, we suggest that the use of *S. patens* should be promoted in the food industry. This recommendation is supported by a previous report in which *S. patens* could reduce serum lipid levels in rats with hyperlipidemia induced by a high-cholesterol diet.[28] Further experiments are ongoing to demonstrate the effect of *S. patens* on the blood glucose level in experimental animals.

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Notes

The authors declare no competing financial interest.

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