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The neuroprotective activities and antioxidant activities of the polysaccharides from *Saccharina japonica*

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ABSTRACT

The crude polysaccharide (W) was extracted by water from *Saccharina japonica* and five fractions were separated by anion-exchange chromatography. And their chemical constituents, neuroprotective activities and antioxidant activities were studied. It showed that W had the neuroprotective activity while its fractions did not. In addition, the fractions displayed higher activities on hydroxyl-radical scavenging effects and reducing power than these of W. Moreover, it was speculated that the neuroprotective activities of samples were related to the hydroxyl-radical scavenging effect and reducing power while did not relate to superoxide-radical scavenging effect. Finally, it was concluded that some fractions could be good candidate antioxidants in food chemistry owing to the high antioxidant activities and their non-toxic characteristics.

1. Introduction

A type of brown seaweed *Saccharina japonica* is extensively cultured in China, Japan and Korea. The harvest is used for the production of mannitol. However, there are three kinds of bioactive polysaccharides: algin (alginate or alginic acid), fucoidan and laminarin. Compared with algin and laminarin, the structure of fucoidan is relatively complicated. On the aspects of biological activities, Fucoidan exhibits anticoagulant activities [1–5], antioxidant acitivity [6] and protective activity against dopaminergic neuron death [7]. In addition, it was reported [1] that both the molecular weight and the content of galactose correlated with the anticoagulant activities. The degree of sulfation was also related to the antiproliferative activity by Teruya et al. [8]. It was also shown [9] that the pattern of linkage involved in the anticancer activity. Moreover, the chemical structures of fucoidan also differ from species to species and also vary with the harvest time, the location of algae, extractions and/or purification methods.

The prevalence of neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's (PD) is increasing along the population ages. It influenced 2% of the population aged greater than 60 years. There are few reports about the application of fucoidan in curing the neurodegenerative diseases. It was shown [10] that fucoidan exhibited a neuroprotective effect on H_2O_2-induced apoptosis in PC 12 cells via activation of the PI3K/Akt pathway. In addition, it was reported in our previous study [7] that fucoidan could protect brain function in a MPTP-induced neurotoxicity model of PD through its antioxidative activity. Antioxidant activities are the most common way to determine the biological activities of polysaccharides.

In order to develop new medicines for neurodegenerative diseases, the mechanisms of the neuroprotective effect of W are necessary to be elucidated. Thus the relationship between neuroprotective activity and antioxidant activities of W and its fractions was characterized in this study.

2. Materials and methods

2.1. Materials

The brown algae *S. japonica* was collected in Rongcheng, Qingdao, China, in April 2012. The standards {L-fucose (Fuc), D-galactose (Gal), D-mannose (Man), D-glucuronic acid (GlcA), L-rhamnose monohydrate (Rha), D-xylose (Xyl) and D-glucose (Glc)} were purchased from Sigma. 3-Methyl-1-phenyl-2-pyrazolin-5-one (PMP) (99%) was obtained from Aldrich Chemistry.

2.2. Extraction and preparations of polysaccharides and degraded polysaccharides

Algae (100 g) were cut into pieces. The crude fucoidan was extracted from the algae with 0.1 M HCl (2 L) at room temperature

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for 3 h. Then the algae residue was dry in the air. The crude polysaccharide (W) was extracted with hot water (2 L) for 2 h from the dried algae residue. Further elimination of algin was achieved by using 20% ethanol with $MgCl_2$ (0.05 M/L). After removing the algin, the supernatant fluid was ultra-filtered. Finally, the dialysate was concentrated and W was obtained by ethanol precipitation. Later, W was separated by anion-exchange chromatography on a DEAE-Bio Gel agarose FF (12 cm × 70 cm) column with elution by 0.2 M NaCl (35 L) (W0.2), 0.3 M NaCl (35 L) (W0.3), 0.5 M NaCl (35 L) (W0.5), 1 M NaCl (35 L) (W1) and 2 M NaCl (35 L) (W2), respectively. Then, all fractions were ultra-filtered, concentrated and precipitated with ethanol.

2.3. Compositional analysis

The sulfated content was performed by ion chromatography on Shodex IC SI-52 4E column (4.0 mm × 250 mm) eluted with 3.6 mM Na_2CO_3 at a flow rate of 0.8 mL/min at 45 °C. The molar ratio of monosaccharide composition and the content of fucose were followed by Zhang et al. [11]. Briefly, polysaccharides (10 mg/mL) were hydrolyzed in trifluoroacetic acid (2 M) under nitrogen atmosphere for 4 h at 110 °C. Then, the hydrolyzed mixture was neutralized to pH 7 with sodium hydroxide. Later the mixture was converted into its PMP derivatives and separated by HPLC chromatography on YMC Pack ODS AQ column (4.6 mm × 250 mm). Uronic acid (UA) was determined by a modified carbazole method [12]. Molecular weight was determined by GPC-HPLC on TSK gel PWxl 3000 column (7 μm, 7.8 mm × 300 mm) eluted with 0.05 M Na_2SO_4 at a flow rate of 0.5 mL/min at 40 °C with refractive index detection. Ten different molecular weight dextrans purchased from the National Institute for the Control of Pharmaceutical and Biological Products (China) were used as standard.

2.4. IR spectroscopy

IR spectra were determined on a Nicolet-360 FTIR spectrometer (36 scans, at a resolution of 6 cm^{-1}) between 400 and 4000 cm^{-1} using powders pressed into KBr pellets.

2.5. Cell culture and MTT assay

The substantia nigra/neuroblastoma hybrid cell lines (MES 23.5) were maintained in Dulbecco's modified Eagle's medium (Gibco Industries, Inc. (Auckland, New Zealand)), supplemented with 5% fetal bovine serum (Gibco Industries, Inc. (Auckland, New Zealand)), 100 units/mL penicillin–streptomycin (Gibco Industries, Inc. (Auckland, New Zealand)) in an atmosphere of 5% CO_2 at 37 °C. Then MES 23.5 cells (200 μL) were seeded in a 96-well plate at a density of 2×10^5 cells/wells for 24 h prior to experimentation. Subsequently, they were divided into the following three groups: (1) control group: control cells were treated in a serum-free medium for 24 h. (2) 6-OHDA group: cells were treated by 6-OHDA (100 μM) (Sigma–Aldrich (St. Louis, MO, USA)) in a serum-free medium for 24 h; (3) experimental groups: cells were treated by 6-OHDA (100 μM) and polysaccharides at the different concentrations (0.5 mg/mL and 0.05 mg/mL) in a serum-free medium for 24 h. Following the removal of medium from the wells, 10 μL of MTT (5 mg/mL suspended in 0.01 M PBS) was added to each well. After 4 h of incubation in a atmosphere of 5% CO_2 at 37 °C, dimethyl sulfoxide (DMSO) (200 μL) was added before the absorbance was measured at 490 nm. The following equation was used to calculate cell vitality: cell vitality (%) = $(A_{sample} - A_{blank})/(A_{control} - A_{blank}) \times 100$.

2.6. Investigation of antioxidant activity

2.6.1. The activity of scavenging hydroxyl radical

The ability of the extracts to scavenge hydroxyl radical was determined according to the modified method [13]. Briefly speaking, 2 mmol/L EDTA–Fe (0.5 mL), sodium phosphate buffer (150 mM, pH = 7.4, 1 mL), crocus (dissolved in above sodium phosphate buffer, 1 mL), 3% H_2O_2 were added sequentially into the solutions (1 mL) with various concentrations of samples. Then the mixtures were incubated at 37 °C for 30 min and detected the absorbance at 520 nm. In the control, distilled water substituted for samples and sodium phosphate buffer substituted as H_2O_2. The scavenging effect (percentage) was calculated using the following equation: scavenging effect (%) = $A_{sample}/A_{control} \times 100$.

2.6.2. The reducing power

The reducing power assay was determined according to the modified method [14]. Briefly speaking, 1.25 mL potassium ferricyanide (1%, w/v) was added into the solutions (1 mL) with different concentrations of samples. Then the mixtures were incubated at 50 °C for 20 min. Later, 2.5 mL trichloroacetic acid (TCA) (10%, w/v) was added into the mixture to end the reaction. Finally, 1.5 mL $FeCl_3$ (0.1%, w/v) was added and the solutions were detected the absorbance at 700 nm.

2.6.3. The activity of scavenging superoxide radical

Superoxide radical assay was determined according to the modified method [6,15]. Briefly speaking, 0.5 mL nicotinamide adenine dinucleotide-reduced (NADH) (0.0365%, w/v), 0.5 mL nitro blue tetrazolium (NBT) (0.0246%, w/v), 0.5 mL phenazine methosulfate (PMS) (0.002%, w/v) were added sequentially into the Tris–HCl buffer (16 mmol/L, pH = 8.0) (3 mL) with different concentrations of samples. Then the solutions were detected the absorbance at 560 nm. In the control, Tris–HCl buffer substituted for samples. The scavenging effect (percentage) was calculated using the following equation: scavenging effect (%) = $(1 - A_{sample}/A_{control}) \times 100$.

2.7. Statistical analysis

All data were shown as mean ± standard deviation (SD). Statistical differences between the experimental groups were determined by one way ANOVA, and differences were considered to be statistically significant if $P < 0.05$. All computations were done by statistical software (SPSS 16.0).

3. Results and discussion

3.1. Extraction and separations of fucoidans and polysaccharides

The yield of W was about 2.1%. Chemical compositions (Table 1) showed that W contained low contents of Fuc and sulfate while it was consisting of high content of UA. In addition, W also had substantial contents of other monosaccharides, especially for Man, GlcA, Gal and Xyl. Thus it was hypothesized that W might consist of fucogalactan, fucoglucuronomannan or fucoglucuronan, which were reported [16–18]. And the chemical compositions of all samples were summarized in Table 1. In addition, their molecular weights were 3.4, 8.3, 15.5, 18.2 and 20.5 kDa (not a symmetrical peak), respectively. The content of sulfate was increasing along with the content of Fuc. And W0.3 had the highest content of UA. Moreover, Fig. 1 shows the IR spectra of W and its fractions. The band at 1610 cm^{-1} was due to the bending vibration of water while intense and broad band at 1250 cm^{-1} of W0.5 and W1 (other fractions did not obviously show this band because of the insufficient amounts, except W2) were attributed to asymmetric O=S=O stretching vibration of sulfates esters. In addition, the band at about