Carbohydrate Polymers 82 (2010) 118–121

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Extraction of the polysaccharides from five algae and their potential antioxidant activity *in vitro*

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ARTICLE INFO

Article history:
Received 19 March 2010
Received in revised form 1 April 2010
Accepted 13 April 2010
Available online 20 April 2010

Keywords:
Polysaccharide
Algae
Antioxidant activities
In vitro

ABSTRACT

The sulfated polysaccharides extracted from algae possess excellent antioxidant activities. In this study, we prepared five polysaccharides extracted from five algae including one brown alga *Laminaria japonica*, one red alga *Porphyra haitanensis* and three green algae *Ulva pertusa*, *Enteromorpha linza* and *Bryopsis plumose*. And then the antioxidant activities of all the samples were investigated including scavenging effects of superoxide and hydroxyl radicals, and reducing power. The chemical analysis and FT-IR spectrum showed these extracts were polysaccharides. And in addition, we found that certain polysaccharide exhibited stronger antioxidant activity in certain antioxidant activity. Factors effecting and attributing to radical scavenging effect need to be further studied.

1. Introduction

In aerobic organisms the energy needed to fuel biological functions is produced in the mitochondria via the electron transport chain. In addition to energy, reactive oxygen species (ROS) which have the potential to cause cellular damage are produced. ROS can damage DNA, RNA and proteins which theoretically contribute to the physiology of ageing (Patel, Cornwell, & Darley-USMAR, 1999). In order to reduce damage to the human body and prolong the storage stability of foods, synthetic antioxidants are used for industrial processing. In general, the natural antioxidants mainly make up many compounds including phenolic, nitrogen compounds and carotenoids (Nandita & Rajini, 2004). In recent years, many marine resources have attracted attention in the search for bioactive compounds to develop new drugs and health foods (Nagai & Yukimoto, 2003).

Polysaccharide, made up of many monosaccharides joined together by glycosidic bonds, possess marked immunological properties ranging from nonspecific stimulation of host immune system, resulting in anti-tumor, anti-viral, and anti-infective effects, to antioxidant, anti-mutagenic or hematopoietic activity (Bohn & BeMiller, 1995; Kennedy & White, 1983; Kennedy, 1989). The high potential for exploiting these natural biopolymers with their broad range of structural, functional and physicochemical properties, in various applications has provided the stimulus for the search for new or modified polysaccharides (Geresh, Dawadi, & Arad, Malis, 2000). Recently, various physiological chemistry effects of polysaccharide from seaweeds have been verified and the interest on sea algae inducing bioactive materials has been increased. In this study, five different polysaccharides were extracted from five algae, *Ulva pertusa*, *Laminaria japonica*, *Enteromorpha linza*, *Bryopsis plumose* and *Porphyra haitanensis*. These five seaweeds were commonly cultured on the coast of China. And they are on behalf of three specials, green alga, red alga and brown alga. Then their antioxidant activities *in vitro* were determined, including superoxide and hydroxyl radical scavenging effects, and reducing power. And then we evaluated their antioxidant activities *in vitro* and characterized the relationship between antioxidant activity and chemical characteristics.

2. Materials and methods

2.1. Materials

Ulva pertusa, *Laminaria japonica*, *Enteromorpha linza* and *Bryopsis plumose* were collected on the coast of Qingdao, China. *Porphyra haitanensis* was collected on the coast of Putian, Chana. The algae were washed; air dried and kept in plastic bags at room temperature cultured.

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doi:10.1016/j.carbpol.2010.04.031

Table 1
Different conditions of extraction from algae in water.

Polysaccharides	Seaweeds	Amount of water (fold)	Temperature (°C)	Time (h)
UP	*Ulva pertusa*	40	125	4
LP	*Laminaria japonica*	15	120	3
EP	*Enteromorpha linza*	40	115	3
BP	*Bryopsis plumosa*	40	115	3
PP	*Porphyra haitanensis*	40	120	4

Nitro blue tetrazolium (NBT), phenazine methosulfate (PMS) and nicotinamide adenine dinucleotide-reduced (NADH), were purchased from Sigma Chemical Co. Ascorbic acid, sodium citrate and other reagents were of analytical grade. Dialysis membranes were produced by Spectrum Co., and molecular weight was cut off at 3600 Da.

2.2. Analytical methods

Sulfate content was determined by barium chloride–gelatin method (Kawai, Seno, & Anno, 1969). Total sugar content was determined by phenol–sulfuric acid method (Dubois, Gillis, Hamilton, Rebers, & Smith, 1956). Uronic acid was estimated in a modified carbazole method using d-glucuronic acid as standard (Bitter & Muir, 1962). Infrared spectrums were measured by a Nicolet Magna-Avatar 360 with KBr disks.

Neutral sugar analysis was determined by high performance liquid chromatography (HPLC). Briefly, the polysaccharides were hydrated and then derivated with PMP. The derivatives were analyzed on HPLC. Chromatographic conditions were generally as follows: column, YMC-Pack ODS-AQ (250 mm × 4.6 mm, 5 μm); temperature, 25 °C; solvent A, 0.4% triethylamine in 20 mM ammonium acetate buffer solution (pH 6.30 with acetic acid)–acetonitrile (9:1); solvent B, 0.4% triethylamine in 20 mM ammonium acetate buffer solution (pH 6.30 with acetic acid)–acetonitrile (4:6); gradient, 10–14% in 9 min, 14–64% from 9 to 30 min, 64% during the next 5 min at 1 mL/min. The eluate was monitored at 245 nm.

Molecular weight of all samples was determined by HP-GPC on a Waters 515 GPC system at 35 °C, where 0.7% Na_2SO_4 solution was used as mobile phase with a flow rate of 0.5 mL/min. TSK G3000 column (300 mm × 7.8 mm) and 2140 refractive index detector was used. A series of different molecular weight dextrans purchased from the National Institute for the Control of Pharmaceutical and Biological Products (China) were used as standard.

2.3. Preparation of natural polysaccharides

In order to obtain high yield of polysaccharides, different methods were applied during the proceeding of extraction from the different algae. Generally, 100 g dry algae were cut roughly and autoclaved in water at 115–125 °C for 3–4 h. The hot aqueous solution was separated by successive filtration with gauze and siliceous earth. The solution was dialyzed against tap water for 48 h and against distilled water for 48 h, and then concentrated under reduced pressure. The polysaccharides were precipitated by the addition of 75% (v/v) ethanol. The resultant precipitate was washed three times with dry ethanol, and then lyophilized to give polysaccharide. Different extract conditions were listed in Table 1.

2.4. Antioxidant activity

2.4.1. Superoxide radical assay

The superoxide radical scavenging abilities of all samples were assessed by the modified method of Zhang et al. (2009). In this experiment, superoxide anion radicals were generated in 4.5 mL Tris–HCl buffer (16 mM, pH 8.0) containing 0.5 mL of NBT (300 μM) solution, 0.5 mL of NADH (468 μM) solution and one sample (0.5–50.0 μg/mL). The reaction was started by adding 0.5 mL of PMS (60 μM) solution to the mixture. The reaction mixture was incubated at room temperature for 5 min and the absorbance was read at 560 nm by a spectrophotometer against blank samples. Decreased absorbance of the reaction mixture indicated increased superoxide anion-scavenging activity. The capability of scavenging the superoxide anion radicals was calculated using the following equation:

$$\text{Scavenging effect}(\%) = \left(\frac{1 - A_{\text{sample}}\,560}{A_{\text{control}}\,560}\right) \times 100$$

where A_{control} 560 is the absorbance of the control (Tris–HCl buffer, instead of sample).

2.4.2. Hydroxyl radical assay

The reaction mixture, containing all different derivatives (0.6–7.0 mg/mL), was incubated with EDTA–Fe^{2+} (2.0 mM), saffron (360 μg/mL), and H_2O_2 (3%) in potassium phosphate buffer (150 mM, pH 7.4), and was incubated for 30 min at 37 °C (Wang et al., 1994). The absorbance was read at 520 nm against a blank. Hydroxyl radical bleached the saffron, so decreased absorbance of the reaction mixture indicated a decrease in hydroxyl radical scavenging ability. The capability of scavenging hydroxyl radical was calculated using the following equation:

$$\text{Scavenging effect}(\%) = \left[\frac{A_0 - A_1}{A_0}\right] \times 100$$

where A_0 is the absorbance of the control (without samples) and A_1 is the absorbance of the mixture containing samples.

2.4.3. Reducing power assay

The reducing power was determined as described previously by Zhang et al. (2009). Briefly, 1.0 mL of different concentration of samples (0.47–6.0 mg/mL) in phosphate buffer (0.2 M, pH 6.6) was mixed with 1.0 mL of potassium ferricyanide (1%, w/v), and was incubated at 50 °C for 20 min. Afterwards, 2.0 mL of trichloroacetic acid (10%, w/v) was added to the mixture to terminate the reaction. Then the solution was mixed with 1.2 mL ferric chloride (0.1%, w/v) and the absorbance was measured at 700 nm. Increased absorbance of reaction mixture indicated increased reducing power.

3. Results and discussion

3.1. Chemical analysis

The polysaccharides extracted from five algae *Ulva pertusa*, *Laminaria japonica*, *Enteromorpha linza*, *Bryopsis plumose* and *Porphyra haitanensis* were named as UP, LP, EP, BP and PP. As these polysaccharides have different degree of the resolution in water at RT, the different extract conditions were applied.

The chemical composition of all the samples was given in Table 2. From the table, the composition of total sugar in PP was the most in these five samples, and that in UP was the least. Correspondingly, PP had the least uronic acids and UP had the most. And what is more, for LP the sulfate content was 25.9, which was

Table 2
The chemical composition and characterizations of all the samples.

Samples	Total sugar (%)	Sulfate (%)	Uronic acids	Neutral sugar (mole ratio)[a]							MW (kD)
				Gal	Fuc	Glc	Ara	Rha	Xyl	Man	
UP	33.4	19.9	19.2	0.01	0	0.29	0.04	1.00	0.49	0.06	151
LP	66.7	25.9	8.45	0.39	1.00	0.03	0.09	0.05	0.02	0.01	250
EP	47.9	16.2	11.9	0.04	0	0.03	0	1.00	0.36	0.04	200
BP	41.8	7.56	7.73	1.00	0.23	0.11	0.72	0.10	0	0.17	227
PP	78.9	17.7	0	1.00	0.05	0	0	0	0.07	0	159

[a] Gal: galactose; Fuc: fucose; Glc: glucose; Ara: arabinose; Rha: rhamnose; Xyl: xylose; Man: mannose.

Table 3
The symbols and IR spectrum data of five polysaccharides.

Samples	IR (KBr) (cm^{-1})
UP	3446, 1641, 1256, 1052, 847
LP	3435, 1643, 1255, 1031, 850
EP	3421, 1647, 1250, 1052, 849
BP	3420, 1650, 1220, 1073, 820
PP	3420, 1646, 1225, 1073, 933, 817

greatly more than that content in BP. For the neutral sugar, there were three monosaccharide units at least in every polysaccharide. Among these five samples: rhamnose was the main sugar unit in UP and EP; fucose was the main sugar unit in LP; galactose was the main sugar unit in BP and PP. Beside the main sugar unit, all the other units were in a small quantity, but especially in BP Arabinose was in a more quantity. The molecular weights of all the samples were large, which lead to the lower degree of solution.

The FT-IR spectrum of the products was shown in Table 3. Infrared spectroscopy analysis indicated that all the three samples showed typical peaks of polysaccharide. The peaks at 820–850, 1220–1260 and 1640–1650 cm^{-1} are caused by the bending vibration of C–O–S of sulfate in axial position, the stretching vibration of S–O of sulfate, and C–O of uronic acids, respectively. Signals at 3420–3450 and 1050–1070 cm^{-1} correspond to stretching vibration of O–H and C–O, respectively. In PP, the weak peak at 933 cm^{-1} was due to the 3,6-anhydrogalactose unites in the polysaccharide.

3.2. Superoxide radical assay

The superoxide radical ($^{\bullet}O^{-2}$) was a highly toxic species that was generated in a PMS/NADH system for being assayed in the reduction of NBT (Banerjee, Dasgupta, & De, 2005). Fig. 1 depicted the inhibitory effect on the superoxide radical of all the samples. From the figure, all the samples showed significant scavenging effects except the sample PP, the inhibitory effect of which was only 68.2% at the concentration of 0.15 mg/mL.

It was reported that addition of electron-withdrawing groups to the pyrrole enhanced antioxidant activity (Yanagimoto, Lee, Ochi, & Shibamoto, 2002). So the presence of the sulfated group could increase the activity of scavenging radicals. Xing et al. (2005) reported that scavenging activity of vitamin C for superoxide radical was 68.2% at 2.0 mg/mL. Compared to this result, all samples had stronger scavenging activity for superoxide radical than vitamin C. Although superoxide was a relatively weak oxidant, it decomposed to form stronger, reactive oxidative species, such as singlet oxygen and hydroxyl radicals, which initiate peroxidation of lipids. Furthermore, superoxides were also known to indirectly initiate lipid peroxidation as a result of H_2O_2 formation, creating precursors of hydroxyl radicals (Dahl and Richardson, 1978). Based on this, the antioxidant activities of all the samples were also related to their ability to scavenge superoxide radical.

3.3. Hydroxyl radical assay

The hydroxyl radical, known to be generated through the Fenton reaction in this system, was scavenged by polysaccharide samples. The scavenging effect of all samples was shown in Fig. 2. For all the samples, the effects of scavenging hydroxyl radicals were in a concentration-dependent manner. Among the five samples, UP, EP and PP showed excellent scavenging effects. But LP and BP had the bad effects even at the high concentration.

For hydroxyl radical, there were two types of antioxidation mechanism; one suppresses the generation of the hydroxyl radical, and the other scavenges the hydroxyl radicals generated. In the former, the antioxidant activity may ligate to the metal ions which react with H_2O_2 to give the metal complexes. The metal complexes thus formed cannot further react with H_2O_2 to give hydroxyl radicals (Ueda, Saito, Shimazu, & Ozawa, 1996). Iron, a transition metal, is capable of generating free radicals from peroxides by the Fenton reaction and is implicated in many diseases. Fe^{2+} has also been shown to produce oxyradicals and lipid peroxidation, and reduction of Fe^{2+} concentrations in the Fenton reaction would protect

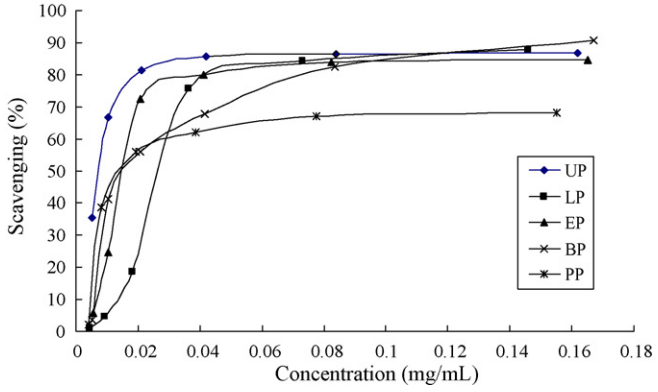

Fig. 1. Scavenging effects of the samples on superoxide radical. Values are means ± S.D. ($n = 3$).

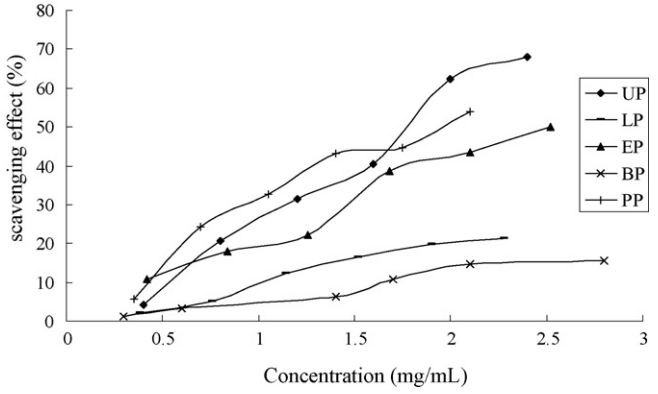

Fig. 2. Scavenging effects of the samples on hydroxyl radical. Values are means ± S.D. ($n = 3$).

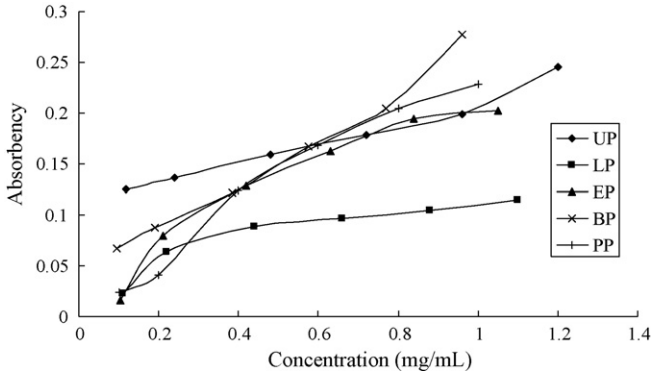

Fig. 3. Reducing power assay of the samples. Values are means ± S.D. ($n = 3$).

against oxidative damage. In the present study, sulfate group had high nucleophilic characteristic and could chelate with metal ion, so the hydroxyl radical scavenging activities of the four samples were stronger than BP. The mechanism of different derivatives on the hydroxyl radicals needs to be further investigation.

3.4. Reducing power assay

The reducing power of all samples was shown in Fig. 3. As shown in the figure, the reducing power of the samples correlated well with increasing concentrations. However, the reducing powers of PP and UP were 0.28 and 0.24 at the concentration of 0.95 and 1.20 mg/mL, respectively. In contrast, the reducing power of LP was only 0.12 at 1.84 mg/mL because of the slow rate of increasing power with increasing concentration.

It has been previously reported that there was a direct correlation between antioxidant activities and reducing power of certain plant extracts (Duh, Du, & Yen, 1999). The reducing properties are generally associated with the presence of reductant, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Gordon, 1990). Reductant is also reported to react with certain precursors of peroxide, thus preventing peroxide formation. In this assay, UP, EP, BP and PP showed excellent reducing power except of LP, sulfate of which was the most. The reason was probably that the ability of donating a hydrogen atom declined because of the decreases of OH.

4. Conclusion

The results of the present work indicated that all samples possessed antioxidant activities in certain assays. These five samples had the strongest radical scavenging effect and may had a use as a possible supplement in the food and pharmaceutical industries. The radical scavenging effect was stable at high temperatures so that these samples may be used as resources of medicine. However, factors effecting and attributing to radical scavenging effect need to be further studied.

Acknowledgements

This work was supported by the Department of Science and Technology of Shandong Province (2007BS07004). We are grateful to Prof. Zuhong Xu and Prof. Zhien Li for their help in this experiment.

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