Food Chemistry 160 (2014) 1–7

Contents lists available at ScienceDirect

Food Chemistry

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

Characterization and antioxidant activities of degraded polysaccharides from two marine Chrysophyta

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ARTICLE INFO

Article history:
Received 13 April 2013
Received in revised form 17 February 2014
Accepted 12 March 2014
Available online 22 March 2014

Keywords:
Marine microalgae
Polysaccharide
Degradation
Physicochemical property
Antioxidant

ABSTRACT

Water-soluble polysaccharides from *Pavlova viridis* and *Sarcinochrysis marina* Geitler (P_0 and S_0, respectively) and their degradation fragments (P_1, P_2, S_1, S_2 and S_3) were screened for their antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydroxyl-radical (OH) scavenging, lipid peroxidation (LPO) inhibition and the mouse red blood cells (RBCs) hemolysis assay. The physicochemical properties of the polysaccharides were also determined. Chemical analysis showed the presence of sulfate groups and uronic acids. Degradation increased the sulfate group content, but also, in part, damaged the uronic acids. FTIR spectroscopy showed that P_0 and S_0 had β-pyranose and α-pyranose configurations, respectively. The low molecular weight fragments after degradation exhibited higher antioxidant capacities, of which P_2 and S_3 showed the strongest antioxidant activity in the given assay system. The half-maximal inhibitory concentration (IC_{50}) values of P_2 on DPPH, OH, LPO and RBCs hemolysis assays were 0.45, 0.42, 0.88, and 1.51 mg/ml, respectively, and the corresponding IC_{50} values of S_3 were 0.41, 0.41, 0.79, and 1.04 mg/ml, respectively. All the polysaccharide fragments evoked a significant dose dependent inhibitory effect or scavenging ability. Altogether, these results suggest that the polysaccharide of two marine Chrysophyta could be considered as a potential antioxidant.

1. Introduction

Reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), the superoxide anion (O_2^-), and hydroxyl radicals ($OH^\cdot$), may be the primary cause of biomolecular oxidation. They may cause significant damage to cell structure, contributing to lipid peroxidation or the formation of DNA adducts that cause cancer-promoting mutations or cell death (Cavas & Yurdakoc, 2005; Cespecles, EI-Hafidi, Pavon, & Alarcon, 2008). Antioxidants are effective in protecting living organisms against oxidative damage caused by ROS. Therefore, commercial antioxidants, especially safe and inexpensive supplements of natural origin, have been in high demand. Marine algae, including marine microalgae, have attracted attention in the search for natural antioxidants to develop new medicinal and functional food ingredients, due to their rich sources of antioxidant compounds with potential free radical scavenging activity. Some antioxidants from various species of seaweeds have been reported in recent years, and the most current studies focused on the detection of antioxidant activity of the total phenolic compounds, crude extracts obtained through organic solvents (methanol or ethanol) and the solvent subfractions from macroalgae (Cho, Lee, Kang, Won, & You, 2011; Ganesan, Kumar, & Bhaskar, 2008; Kumar, Ganesan, Rao, & Subba, 2008; Lopez, Rico, Rivero, & Tangil, 2011; Wang et al., 2010; Wang, Jonsdottir, & Olafsdottir, 2009; Wang, Zhang, Duan, & Li, 2009). Among the bioactive constituents, polysaccharides from natural sources are found to be effective, non-toxic substances with a wide variety of pharmacological activities, such as immunomodulating, antitumour, anti-imflammatory and antioxidant (Ananthi et al., 2010; Liu, Chang, Zhang, Zhang, & Li, 2012). In recent years, several reports have revealed that algal polysaccharides exhibit strong free-radical scavenging activity and can be used as antioxidants for the prevention of oxidative damage in living organisms (Ananthi et al., 2010; Kim, Choi, Athukorala, Senevirathne, & Rha, 2007; Liu et al., 2012; Souza et al., 2012, 2007). Microalgae can easily be cultivated on a large scale, and have been widely used as functional food ingredients or in aquatic animal feed directly, such as common species *Spirulina*, *Chlorella*, and *chrysophyceae*. However, little attention has been given to the investigation of the antioxidant potential of polysaccharides from microalgae, although more bioactive compounds from microalgae have been developed (Plaza, Herrero, Cifuentes, & Ibanez, 2009).

Previous work has revealed the apparent antioxidant activities of polysaccharides from the microalga, *Porphyridium cruentum*,

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http://dx.doi.org/10.1016/j.foodchem.2014.03.067
0308-8146/

and verified that the molecular weight (MW) of the polysaccharides is related to their biological activities (Sun, Wang, & Shi, 2009). Because of the heterogeneity of MW and the poor water solubility, the undegraded polysaccharides are usually not readily absorbed, and have poor bioactivity. Moreover, high-MW polysaccharides directly injected into the body may be toxic and this could limit their application (Mazumder, Ghosal, & Pujol, 2002). Hence, oligosaccharides or low-MW polysaccharide fragments obtained by means of chemical degradation could be used more advantageously to reduce immunogenicity and improve the biological activity (Chen, Zhang, & Wang, 2007).

The previous results encouraged us to consider other species of microalgae. In the present study, the physicochemical properties and antioxidant activities of polysaccharides and the low-MW degraded fragments isolated from marine microalgae *Pavlova viridis* and *Sarcinochrysis marina* Geitler, two kinds of chrysophytes, were evaluated after water extraction, degradation and purification with gel column chromatography. The objectives of this work were to determine the antioxidant activity and study the relationship between bioactivity and physicochemical properties or the molecular weight. The findings of the present report may contribute to a rational basis for the use of microalgae polysaccharides in possible diseases therapy or a natural antioxidant as a food additive, as well as appear useful for further research aiming to isolate polysaccharides from other microalgae.

2. Materials and methods

2.1. Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), Sepharose-6B, D-galactose, glucuronic acid, standard dextrans, thiobarbituric acid (TBA) and lecithin were purchased from Sigma Chemical Co. (St. Louis, Mo, USA). Trichloroacetic acid (TCA), hydrogen peroxide (H_2O_2), carbazole and phenanthroline were purchased from Shanghai Chemical Reagents Co. (Shanghai, China). All chemicals and other reagents were of analytical grade.

2.2. Preparation of algal powder and polysaccharide from the two microalgae

The original *P. viridis* and *S. marina* Geitler cells were provided from the Marine Alga Culture Collection of Ocean University of China. In this experiment, the cells cultured in suspending medium were collected by centrifugation at 4000 rpm for 15 min, and then the sediment was vacuum freeze-dried for 48 h to obtain the algal powder.

The microalgal powders were extracted with 5 volumes of distilled water and then freeze-thawed for 3 cycles. Afterward, they were extracted with distilled water (3 times) by ultrasonication at 300 W, 65 °C for 2 h. After centrifugation at 4000 rpm for 15 min, the supernatant was collected and concentrated to a third of the original volume using a rotary evaporator. Trichloroacetic acid (TCA, 10%) was added to remove free protein. After further centrifugation at 4000 rpm for 15 min, the supernatant was dialyzed for 48 h with flowing water, and 24 h with distilled water. Ethanol (95%) was added, and the precipitate produced, the polysaccharides of *P. viridis* and *S. marina* (P_0 and S_0, respectively), were vacuum freeze-dried and then washed with anhydrous ethanol. P_0 and S_0 were subjected to degradation by a H_2O_2–vitamin C (Vc) system aided by ultrasonication, according to a procedure by Zhang, Zhao, and Niu (2005). The degradation condition 1 was a concentration of 8 mM H_2O_2 and Vc 8, ultrasonication action intensity 300 W, at 50 °C for 2.5 h; condition 2 was 12 mM H_2O_2 and Vc 12, ultrasonication action intensity 300 W, at 60 °C for 3.5 h. The original polysaccharides and the degraded fragments (0.01 M) were purified on a Sepharose 6B gel filtration column (2.2 × 80 cm). The polysaccharides were eluted with 0.9% (w/v) NaCl at a flow rate of 0.6 ml/min. The collected fractions were dialyzed, again, to obtain the relative homogeneous fragments P_0, P_1, P_2, S_0, S_1, S_2 and S_3.

2.3. Determination of physicochemical properties of the polysaccharides

The total sugar content was estimated using the phenol–sulfuric acid method with D-galactose (Sigma, St. Louis, MO, USA) as a standard. The sulfate content was determined by barium sulfate turbidity analysis, according to the method of Zhang (2003). The uronic acid content was measured by the carbazole–sulfuric acid method using glucuronic acid as a standard (Lin, Huang, & Tian, 1999). The monosaccharide components were determined by gas chromatography (GC) using an Agilent 6820 (Agilent, USA) equipped with a flame ionization detector and an SE-54 capillary column (50 m × 0.2 mm × 0.25 μm). Nitrogen was used as the carrier gas and was introduced at a flow rate of 2.2 ml/min. The monosaccharide derivatives were prepared in aldononitrile acetate (Sun et al., 2009). The average MW of the polysaccharides was measured by high-performance gel permeation chromatography using two types of columns in series (TSK-G5000PW 7.5 mm × 300 mm; G4000PW 7.5 mm × 300 mm; Tosoh, Japan) in an Agilent 1100 LC equipped with a ultraviolet (UV) detector and refractive index detector. The operating parameters and calculations of MW were done according to Sun et al. (2009).

2.4. Fourier transform infrared (FTIR) spectroscopy

The IR spectra of the polysaccharides were detected using an FTIR spectrophotometer (Shimadzu IR-400, Japan). The polysaccharide was ground with spectroscopic-grade KBr powder and then pressed into 1 mm pellets. FTIR measurements were in the wavenumber range of 400 and 4000 cm^{-1} using 16 scans (Souza et al., 2012).

2.5. Determination of antioxidant activities

2.5.1. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay

The free-radical scavenging capacity of the polysaccharides were measured by a DPPH assay according to a modification of the method of Souza et al. (2012). DPPH (2 ml of 40 μg/ml in methanol) was added to 2 ml of various doses of the polysaccharides (0.063–1.0 mg/ml) in 10 ml test tubes. The mixture was shaken vigorously, kept at room temperature for 30 min, and then its absorbance was read at 517 nm (A_{517}) using a UV–visible spectrophotometer. The DPPH· scavenging effect was calculated from the following equation:

$$\text{Scavenging effect}\,(\%) = \frac{A_c - A_s}{A_c} \times 100 \quad (1)$$

where A_c was the absorbance of the control (methanol with DPPH solution) and A_s was the absorbance of the polysaccharide sample.

2.5.2. Hydroxyl-radical scavenging activity

The scavenging activity of the microalgal polysaccharides against the hydroxyl radical was investigated using the Fenton reaction. Hydroxyl radicals were generated using the method of Smirnoff and Cumbes (1989) with slight modifications. The reaction mixture contained 1 ml of 9 mM $FeSO_4{\cdot}7\ H_2O$, 1 ml of 9 mM sodium salicylate, 1 ml of 8.8 mM H_2O_2, and varying concentrations of the polysaccharides (0.063–1.0 mg/ml) in sodium phosphate buffer (150 mM, pH 7.4). In the control sample, sodium phosphate buffer replaced H_2O_2. The solutions were incubated at