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International Journal of Biological Macromolecules 37 (2005) 195–199

INTERNATIONAL JOURNAL OF
Biological
Macromolecules
STRUCTURE, FUNCTION AND INTERACTIONS

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Antioxidant activity of different sulfate content derivatives of polysaccharide extracted from *Ulva pertusa* (Chlorophyta) in vitro

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Received 1 September 2005; received in revised form 15 September 2005; accepted 21 October 2005

Abstract

Polysaccharide extracted from *Ulva pertusa* (Chlorophyta) is a group of sulfated heteropolysaccharide; for simplicity, the sulfated polysaccharide is referred to as ulvan in this paper. In this study, different sulfate content ulvans were prepared with sulfur trioxide/*N*,*N*-dimethylformamide (SO_3–DMF) in formamide, and their antioxidant activities were investigated including scavenging activity of superoxide and hydroxyl radicals, reducing power and metal chelating ability. As expected, we obtained several satisfying results, as follows: firstly, high sulfate content ulvans had more effective scavenging activity on hydroxyl radical than natural ulvan. Secondly, comparing with natural ulvan, high sulfate content ulvans exhibited stronger reducing power. Thirdly, HU4 (sulfate content, 30.8%) and HU5 (sulfate content, 32.8%) showed more pronounce chelating ability on ferrous ion at high concentration than other samples.

Keywords: Different sulfate content; Ulvan; Antioxidant activity; Metal chelating ability; Radical scavenging effect; Reducing power

1. Introduction

The green alga, *Ulva pertusa*, is an important food source in many parts of the world. *U. pertusa* is nutritious with low calorie, abundant vitamins, trace elements and dietary fibers [1]. Moreover, it has been used as a drug in traditional Chinese medicine for hyperlipidemia, sunstroke, and urinary diseases, etc. Polysaccharide extracted from *U. pertusa* is a group of heteropolysaccharide, mainly composed of rhamnose, xylose, glucose, glucuronic acid, iduronic acid, and sulfate, with smaller amounts of mannoses, arabinose, and galactose. The mainly repeating disaccharide units are [β-D-GlcpA-(1 → 4)-α-L-Rhap 3s] and [α-L-IdopA-(1 → 4)-α-L-Rhap 3s] [2–5]. For simplicity, these sulfated rhamnoglycuronans are referred to as ulvan in this paper.

Reactive oxygen species (ROS), capable of causing damage to DNA, have been associated with carcinogenesis, coronary heart disease, and many other health problems related to advancing age [6,7]. In order to reduce damage to the human body and prolong the storage stability of foods, synthetic antioxidants are used for industrial processing. The most commonly used antioxidants at the present time are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and *tert*-butylhydroquinone (TBHQ). However, BHA and BHT have been suspected of being responsible for liver damage and carcinogenesis [8]. Thus, it is essential to develop and utilize effective and natural antioxidants so that they can protect the human body from free radicals and retard the progress of many chronic diseases [9,10].

In recent years, many marine resources have attracted attention in the search for bioactive compounds to develop new drugs and health foods. In addition, marine algae are now being considered to be a rich source of antioxidants [11]. Some active

Abbreviations: DMF, *N*,*N*-dimethylformamide; EDTA, ethylene diamine tetra-acetic acid; FA, formamide; $HClSO_3$, chlorosulfonic acid; H_2O_2, hydrogen peroxide; NADH, nicotinamide adenine dinucleotide-reduced; NBT, nitro blue tetrazolium; PMS, phenazine methosulfate; SO_3, sulfur trioxide; TBA, thiobarbituric acid; TCA, trichloroacetic acid

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doi:10.1016/j.ijbiomac.2005.10.008

antioxidant compounds from brown algae were identified as phylopheophytin in *Eisenia bicycli*s (arame) and fucoxantine in *Hijikia fusiformis* (hijiki) [12,13]. Algal polysaccharides have been demonstrated to play an important role as free-radical scavengers in vitro and antioxidants for the prevention of oxidative damage in living organisms [14]. Their activity depends on several structural parameters such as the degree of sulfation (DS), the molecular weight, the sulfation position, type of sugar, and glycosidic branching [15]. Chemical modification of polysaccharides provided an opportunity to obtain new pharmacological agents with possible therapeutic uses [16]. Xing et al. [17] investigated antioxidant activity of chitosan and sulfated chitosans, and they found that sulfated chitosans exhibited stronger antioxidant activity than that of unmodified chitosan, which indicated that modified polysaccharide, such as sulfated chitosan, might have increased antioxidant activity. Regarding the relationship between sulfate content and macrophage stimulating activity of polysaccharides from *P. yezoensis*, it was reported that sulfate groups in the molecules probably contribute to the activity [18].

In the present study, different sulfate content ulvans were prepared with sulfur trioxide/*N*,*N*-dimethylformamide (SO_3–DMF) in formamide and their antioxidant activities in vitro were determined, including scavenging ability of superoxide and hydroxyl radicals, chelating ability of iron ion and reducing power. Moreover, at present, antioxidant activity of different sulfate content ulvans has not been reported.

2. Experimental

2.1. Materials

U. pertusa was collected on the coast of Qingdao, China. Algae were washed, air dried and kept in plastic bags at room temperature before using. Nitro blue tetrazolium (NBT), phenazine methosulfate (PMS), nicotinamide adenine dinucleotide-reduced (NADH), ethylene diamine tetra-acetic acid (EDTA), hydrogen peroxide (H_2O_2), ferrozine, trichloroacetic acid (TCA), thiobarbituric acid (TBA), and deoxyribose (DR) were purchased from Sigma Chemical Co. All 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 using the traditional method of barium chloride–gelatin [19].

2.3. Preparation of natural ulvan

Dry algae (100 g) were cut and autoclaved in 4000 mL of water at 125 °C for 4 h. The hot aqueous solution was separated from the algae residues by successive filtration through gauze and siliceous earth. The solution was dialyzed against tap water for 48 h and against distilled water for 48 h, and then the solution was concentrated to about 1000 mL under reduced pressure. The polysaccharides were precipitated by the addition of 4000 mL of 95% (v/v) ethanol. The resultant was washed three times with ethanol, then dried at 80 °C (mean yield, 22.5%) [20,21].

Table 1
Symbols of ulvan and high sulfate content ulvans in determining antioxidant activity

Symbols	Sulfate content (%)
U	19.5
HU1	23.5
HU2	25.7
HU3	27.9
HU4	30.8
HU5	32.8

2.4. Preparation of different sulfate content ulvans

The sulfation agent, SO_3–DMF, was obtained by dropping 50.0 mL of chlorosulfonic acid ($HClSO_3$) into 300 mL of *N*,*N*-dimethylformamide (DMF) under cooling in an ice-water bath. Dry ulvan (2 g) was added to 80 mL of formamide (FA), and the mixture was stirred at different temperatures (20–100 °C) for 30 min in order to disperse it into solvent. Then SO_3–DMF reagent (5, 15, and 25 mL) was added. After 4 h, the mixture was cooled to room temperature by an ice bath, neutralized with 2 M NaOH solution, and precipitated with 85% ethanol for 24 h. The precipitate was filtered off and washed three times with ethanol, and then was dissolved in 100 mL distilled water. The solution was dialyzed against tap water for 48 h and distilled water for 48 h using 3600 Da Mw cutoff dialysis membranes. The resultant was concentrated and lyophilized to give different sulfate content ulvans [22]. Five sulfated derivatives with different sulfate content were obtained by varying the reaction temperature and the molar ration of ulvan/SO_3–DMF with fixed reaction time (Table 1).

2.5. Antioxidant activity

2.5.1. Superoxide radical assay

The superoxide radical scavenging ability of all different sulfate content ulvans was assessed by the method of Nishimiki et al. [23]. The reaction mixture, containing different sulfate content ulvans (6.0–200 μg/mL), Tris–HCl (16 mM, pH 8.0), NADH (338 μM), NBT (72 μM), and PMS (30 μM), was incubated at room temperature for 5 min and the absorbance was read at 560 nm against a blank. The capability of scavenging to superoxide radical was calculated using the following equation:

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

2.5.2. Hydroxyl radical assay

The reaction mixture, containing all different sulfate content ulvans (0.38–2.0 mg/mL), was incubated with deoxyribose (3.75 mM), EDTA (100 μM), ascorbic acid (100 μM), H_2O_2 (1 mM), and $FeCl_3$ (100 μM) in phosphate buffer (20 mM, pH 7.4) for 60 min at 37 °C [24]. The reaction was terminated by