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Chemical Characterization and Antioxidant Activities of Different Sulfate Content of λ -Carrageenan Fractions from Edible Red Seaweed *Chondrus ocellatus*

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Abstract

In recent years, sulfated polysaccharides from marine algae have been demonstrated to have many activities including antioxidant activities. But whether degraded sulfated polysaccharides from *Chondrus ocellatus* have antioxidant activities, and what effects their sulfate content may have on those activities has not been reported. The aim of this study is to explore the antioxidant activities of λ-carrageenan fractions from edible red seaweed *Chondrus ocellatus*. In the present study, λ-carrageenan from *Chondrus ocellatus* was degraded by microwave, yielding five products of different molecular weights. Three sulfated polysaccharide fractions (CF1, CF2, and CF3) were isolated from one of the degraded λ-carrageenans (PC2) by anion-exchange column chromatography. In this study, the effects of the three polysaccharide fractions on hydroxyl radicals, superoxide radicals, organic free radical DPPH, H_2O_2 induced hemolysis of rat erythrocytes and lipid peroxidation in rat liver microsome were investigated. The results indicated that IR spectrum results indicated that these sulfated polysaccharide fractions had similar structures, but differed in their sulfate content. Chemical analysis indicated that CF1 had the lowest sulfate content (2.9%), while sulfate content in CF2 and CF3 was 7.8% and 37.5% respectively. CF3 had stronger scavenging activity on superoxide radical and hydroxyl radical than the other fractions ($P<0.05$). CF3 had the strongest scavenging activity on DPPH. the highest inhibitor of lipid peroxidation was CF3, with an IC_{50} of 0.087 mg/mL, and inhibitory rate reaching 90% at 1 mg/mL. All of the three fractions exhibited stronger scavenging activity at high concentration. In conclusion, sulfate group content of polysaccharides also effect on their biological activities, and which is important for antioxidant activities.

Keywords: Antioxidant; λ-carrageenan; Different sulfate content; *Chondrus ocellatus*

Introduction

Oxygen-derived free radicals (superoxide and hydroxyl radical) play important roles in the process of ageing and carcinogenesis. They are also related to inflammation, shock, and ischemia/reperfusion injury [2]. In recent years, sulfated polysaccharides from marine algae have been demonstrated to have many activities including antioxidant activities [4]. For instance, sulfated polysaccharides from *Fucus vericulosus* [12]. *Gigartina skottsbergii* and *Schizymenia binderi*, commercial carrageenans, and fucoidan from *Lessonia vadosa* Tamara [1], *Ulva pertusa* [6], *Porphyridium* sp [10] and *Laminaria japonica* [13] have been demonstrated to have antioxidant activities. But whether degraded sulfated polysaccharides from *Chondrus ocellatus* have antioxidant activities, and what effects their sulfate content may have on those activities has not been reported.

Chondrus ocellatus, distributed widely in China and many other parts of the world, is an economically important native algae. All of the algae are edible, and can be used medicinally, primarily to treat chronic constipation, bone fracture. We previously degraded λ-carrageenan by microwave method which yielded five products with different molecular weights. Anti-tumor and immunomodulation activities were investigated [16-18]. In this study, we chose one of the five samples, PC2, from which to separate new fractions. Three sulfated polysaccharide fractions (CF1, CF2 and CF3) were isolated through anion-exchange column chromatography. The antioxidant activities of these sulfated polysaccharide fractions were studied. In addition, the relationship between the sulfate content and antioxidant activities was investigated.

Materials and Methods

Experiment material

Chondrus ocellatus was collected on Taiping Cape, Qingdao, in April, 2003. After picking, the coarse weed was discarded, and the remaining material was washed thoroughly, air-dried, and sealed plastic bags for preservation. The crude polysaccharide from *Chondrus ocellatus* was extracted with hot water, and λ-carrageenans (PC1) were produced by the conventional method [9] with some improvements. Degraded λ-carrageenan (PC2) was then produced by microwave [9].

Fractionation

Further fractionation was achieved by anion-exchange chromatography. The crude polysaccharide was dissolved in distilled water and applied to a column (2.5 × 35 cm) of DEAE-cellulose (Whatman DE52) pre-equilibrated with distilled water. Fractions were prepared by stepwise elution with distilled water, 0.5mol/L NaCl, 1.0mol/L NaCl, 1.5 mol/L NaCl and 2.0 mol/L NaCl solution in turn. The elution was detected by phenol-sulfuric acid method. Each

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elution was collected, dialyzed, concentrated by rotary evaporation, precipitated with ethanol and dried.

Chemical analysis

Sulfate content was determined according to the method of Kawai [5]. Infrared spectra were recorded from polysaccharide powder in KBr pellets on a Nicolet Avatar 360FT-IR spectrometer. UV analysis was conducted on SHIMADZU 1601 UV visible spectrometer by scanning 0.05% aqueous solution of sample in 200-400nm wavelength range.

Neutral sugar was analyzed by GLC. Polysaccharide sample was hydrolyzed in a sealed glass tube with 2 mol/L trifluoroacetic acid for 8 h at 100°C. The acid was removed under reduced pressure by several evaporations with distilled water, then the hydrolysates were converted to acetylated aldononitrile derivatives according to conventional procedures and analyzed by GLC (Agilent 6820) using a AC-20 capillary column (30 m × 0.32 mm ID). As references, the following neutral sugars were converted to their acetylated aldononitrile derivatives and analyzed: rhamnose, fucose, arabinose, xylose, mannose, galactose and glucose.

Determination of antioxidant activity

Hydroxyl radicals were generated by the method of Smirnoff and Cumbes [8] in sodium phosphate buffer (400 mmol/L, pH 7.4), which contained 2.5 mmol/L $FeSO_4$, 2.5 mmol/L sodium salicylate, 6 mmol/L H_2O_2, and varying concentrations of polysaccharides (62.5 μg/mL~2 mg/mL). In the essential control, sodium phosphate buffer replaced H_2O_2. The solutions were incubated at 37°C for 1.5 h, and absorbance at 536 nm was measured.

Superoxide radicals were generated by the self-oxidation method of pyrogallol [19]. The reaction system is: 2.4mL Tris-HCl buffer (pH 8.2), 0.1mL varying concentrations of polysaccharides, 7 mmol/L pyrogallol 0.3 mL, 1drop 10mmol/L HCl to terminate the reaction 4 mins after mixed, then absorbance at 325nm was measured.

Effects of polysaccharides on organic free radical DPPH were assayed by the method of Larrauri [14]. The volume of reaction was 4mL. DPPH was precipitated in a small volume of methanol, and resuspended in 50% ethanol to a concentration of 120 μMol/L. The reaction volume was 0.2 mL polysaccharides solution and 3.8 mL DPPH. After 20 min, and absorbance at 525 nm was measured.

Effects of polysaccharides on H_2O_2 induced hemolysis of rat erythrocytes and lipid peroxidation by rat liver microsomes were assayed according to the method of Zhang [14]. Erythrocytes were separated by centrifugation from blood samples of male Wistar rats and suspended in physiological saline to a final concentration of 0.5%. In 2 mL reaction solution, which contained 1mL erythrocyte suspension, varying concentrations of polysaccharides and 100mM H_2O_2, were incubated at 37°C for 1h, then diluted in 4 volumes of physiological saline, and centrifuged at 1000 rpm for 10 min. The supernatant was measured for absorbance at 414 nm. The essential control contained only erythocyte suspension in 2 mL solution.

Liver microsomes were prepared from male Wister rats according to the method of Zhang [14]. Protein content of microsomes was measured by the Bradford method. The product of microsomal lipid peroxidation was malondialehyde. Microsomes were incubated at 37 °C for 1h with varying concentrations of polysaccharides, 10 μmol/ L$FeSO_4$ and 0.1mmol/L ascorbic acid in 1.0 mL potassium phosphate buffer solution (0.2 mol/L, pH 7.4). The reaction was stopped by 20% trichloroacetic acid (1.0 mL) and 0.67% 2-thiobarbituric acid (1.5mL) in succession, and the solution was then heated at 100°C for 15min. After the precipitated protein was removed by centrifugation, the color reaction of malondialdehyde- thiobarbituric acid complex was detected by measuring absorbance at 532 nm. The positive control did not contain $FeSO_4$ and ascorbic acid.

The percentage of antioxidant activity of the samples was calculated according to the following formula: Inhibition rate (%) $=(A_0-A)/(A_0-A_e)\times100\%$, where A0 is the absorbance of the free radical generation system, A is the absorbance of the test sample, Ae is the absorbance of the positive control.

Statistical analysis

The data presented are means ± SD of three measurements followed by Student's t test. Differences were considered to be statistically significant if $P<0.01$.

Results

Fractionation

The eluting profile of chromatography for polysaccharides was shown in Figure 1.Three fractions were obtained: CF1 from 0.5 mol/L NaCl elution, CF2 from 1.0 mol/L NaCl, and CF3 from 1.5mol/L NaCl. Their yields were 10.2%, 29.5% and 60.3%, respectively. Almost no polysaccharide was detected in distilled water or the 2.0 M NaCl elution.

Chemical analysis

The Infrared absorption spectra of the λ-carrageenan samples from *Chondrus ocellatus* indicated that all the three fractions showed typical λ-carrageenan absorbance comparable to the standard purchased from Sigma Chemical Company (St. Louis, Mo. USA). They all showed a general absorption band at 1250-1270 cm^{-1}, which is attributed to the asymmetric stretching vibration of the sulfate group, but this peak is much weaker in CF1 and CF2. In addition, CF2 and CF3 showed an absorption band at roughly 817 cm^{-1}. The signal was indicative of a sulfate group attached to a primary hydroxyl group. This suggested that the sulfate groups occur at C-6 of galactosyl residues. This peak did not appear in the spectra of CF2. However, CF2 showed a peak at 931.55 cm^{-1}, which indicated the presence of 3,6-anhydrogalactopyranosyl residues, but this peak did not appear in the spectra of CF1 and CF3.

CF1 had the lowest sulfate content (2.9 %), while the sulfate content in CF2 and CF3 was 7.8% and 37.5% respectively.

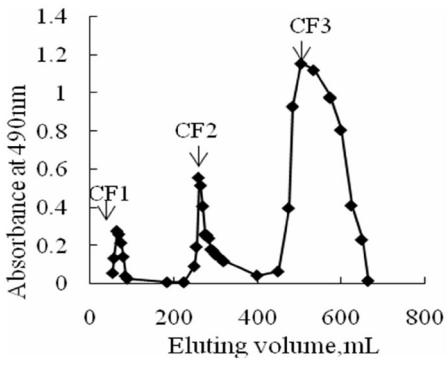

Figure 1: Eluting profile of crude polysaccharide on DEAE-cellulose chromatography. (eluted with 0.5 mol.L^{-1}; 1.0 mol.L^{-1}; 1.5 mol.L^{-1} and 2.0 mol.L^{-1} NaCl, respectively.

The UV spectra of the polysaccharides did not show any peak in the range of 220-300 nm, indicating almost no nucleic acid and protein existed in the samples.

Neutral monosaccharide constitutions of the polysaccharides were analyzed by GLC. For all three samples, galactose was the only sugar unit.

Antioxidant activities

Figure 2 shows the results of hydroxyl radical scavenging activity of polysaccharide samples. All the samples showed hydroxyl radical scavenging activity at high concentration with IC_{50} over 0.1 mg/mL. CF1 and CF2 had similar hydroxyl radical scavenging effect, while the effect of CF3 was much stronger.

Figure 3 shows the superoxide radical scavenging effect of the three polysaccharide fractions. All fractions showed weak superoxide radical scavenging activity. CF3 had stronger scavenging activity on superoxide radical than the other fractions, while CF1 had the weakest effect.

DPPH is one kind of stable organic free radical. The results of DPPH scavenging activity of the three polysaccharide fractions are shown in Figure 4, indicating they all had this activity. The IC_{50} of CF3 was 0.133mg/mL. CF3 had the strongest scavenging activity on DPPH, while CF1 had the weakest effect.

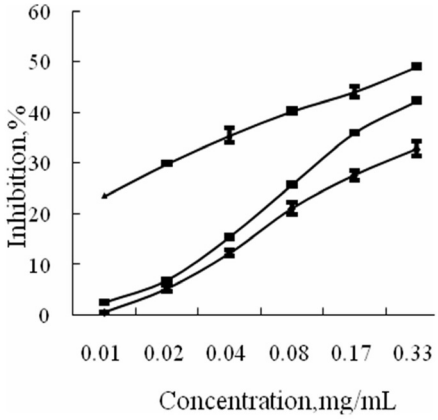

Figure 2: Inhibitory effects of sulfated polysaccharide fractions CF1(♦), CF2(■), and CF3 (▲) on hydroxyl free radicals. Values are means ± SD (n=3).

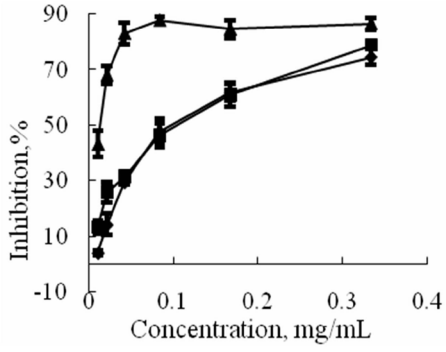

Figure 3: Inhibitory effects of sulfated polysaccharide fractions CF1(♦), CF2(■), and CF3(▲)from *Condrus ocellatus* on super-oxide radicals. Values are means ± SD (n=3).

Savenging effects of the three λ-Carrageenan fractions from *Chondrus ocellatus* on lipid peroxide of rat liver microsome are shown in Figure 5. All showed similar significant effect and the inhibitory rate increase as the concentration of each λ-Carrageenan simple was increased.

Among the three samples, the highest inhibitor of lipid peroxidation was CF3, with an IC_{50} of 0.087 mg/mL, and inhibitory rate reaching 90% at 1 mg/mL.

Figure 6 shows the effects of the three λ-carrageenan species from

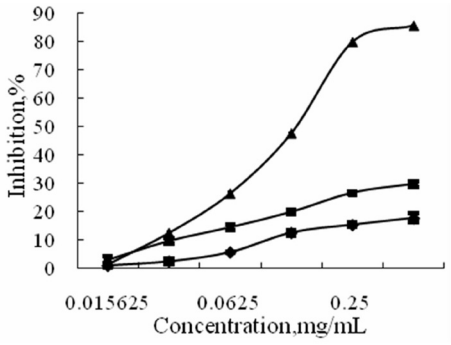

Figure 4: Inhibitory effects of sulfated polysaccharide fractions CF1(♦), CF2(■), and CF3 (▲) from *Condrus ocellatus* on DPPH. Values are means ± SD(n=3).

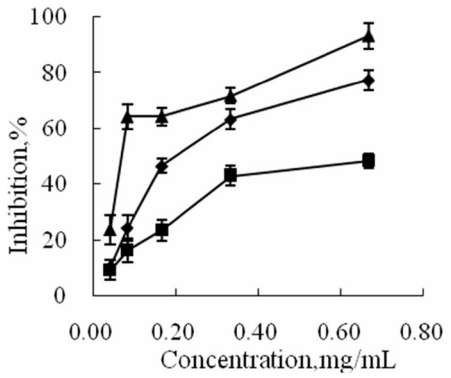

Figure 5: Inhibitory effects of CF1(♦),CF2(■) and CF3(▲) on lipid peroxidation in liver homogenate. Values are means ± SD (n=3).

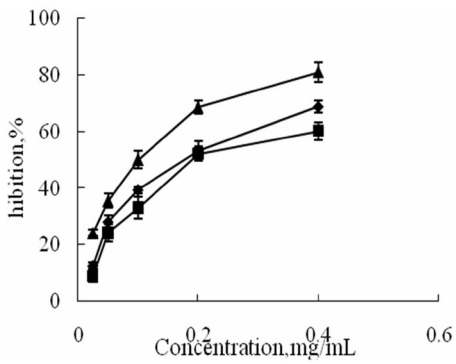

Figure 6: Effects of CF1(♦), CF2(■) and CF3(▲) on RBC hemolysis induced by H_2O_2. Values are means ± SD (n=3).

Chondrus ocellatus on H_2O_2 induced hemolysis of rat erythrocytes. The degree of hemolysis of each λ-carrageenan group were lower than the negative group in each of the five doses. They all exhibited stronger scavenging activity at high concentration. Their IC_{50} were 0.216 0.312 and 0.120mg/mL, respectively. Also, the difference was greatest between each λ-carrageenan group at different concentrations (p<0.01). This means that concentration of λ-carrageenan has significantly effect on the activity.

Discussion

Currently there is growing interest in using natural medicines to avoid the undesirable side-effects of synthetic compounds. In this study, three sulfated polysaccharide fractions (CF1, CF2, and CF3) were isolated from naturally-derived degraded λ-carrageenan, through anion exchange column chromatography. By chemical analysis, we determined that these fractions had the same sugar unit as the original compound, but differed in their relatively high sulfate content.

The three polysaccharide fractions all exhibited antioxidant activities on different degrees. CF3, which had the highest sulfate content, had the strongest scavenging activity on superoxide radical, DPPH, hydroxyl radical and lipid peroxidation in rat liver microsomes. This suggests that sulfate content is important for the antioxidant activities of λ-carrageenan. CF1 and CF2 had similar sulfate content, as well as hydroxyl radical and superoxide radical scavenging effect, but showed little difference in scavenging activity on DPPH. This result was consistent with chemical analysis and IR spectra. According to chemical analysis and IR spectra, CF3 had the highest sulfate content, while CF1 had the lowest sulfate content. CF2 contained a small amount of 3,6-anhydrogalactose. These results indicate that degraded λ-carrageenans are effective antioxidants like other sulfated polysaccharides, and the sulfate content of polysaccharides has a significant effect on their antioxidant activities.

Others have reported on the structure-antioxidant activity relationship of polysaccharides [3,11,7]. They observed that sulfate group content is an important factor affecting the antioxidant activity of sulfated polysaccharides. Qi [6] found that a polysaccharide from *U. pertusa* with low sulfate content and low molecular weight had a stronger reducing power and free radical scavenging effect than other sulfated polysaccharides. Zhao [15] found that the antioxidant activity of sulfated polysaccharides is apparently related not only to molecular weight and sulfated ester content, as previously determined, but also to glucuronic acid and fructose content.

In conclusion, sulfate group content of polysaccharides also effect on their biological activities, and which is important for antioxidant activities. Firmer conclusions require further investigation.

Declaration

We declare that we have no conflict of interest.

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