Antioxidant Properties of Different Molecular Weight Polysaccharides from Seaweed Sargassum Fusiform

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Abstract. This study comparatively analyzed the antioxidant activities of original and three degraded sargassum fusiform polysaccharide fractions, named SFP0, SFP1, SFP2, and SFP3, with molecular weight of 16.2, 8.21, 3.83, and 2.23 kDa, respectively. Results revealed that the scavenging activity of various SFP fractions for hydroxyl, DPPH, and ABTS radicals and the reducing power increased with decreasing molecular weight of SFP within 16.2-2.23 kDa. SFP3, with the lowest molecular weight of about 2.23 kDa, exhibited the strongest radical scavenging capacity. Moreover, the Fe²⁺ chelating ability also increased with decreasing molecular weight of various SFP fractions. The active groups (such as -OSO₃H) of low-molecular-weight polysaccharides was easily exposed and possessed high degree of freedom and low space steric hindrance; hence, their chelating ability increased. These SFP fractions, especially SFP3, may be potential anti-stone drugs.

Introduction

Reactive oxygen species (ROS) in the forms of superoxide anion (•O₂⁻), hydroxyl radical (•OH) and H₂O₂ are produced by metabolic process or external factors [1]. These ROS are the media that cause all kinds of diseases of human body, and although there is antioxidant and repair oxidative damage defense system in human body, these systems are unable to prevent this oxidative damage completely. Therefore, many antioxidant drugs have been synthesized such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tert-Butylhydroquinone (TBHQ). However, many researchers began to study some natural antioxidant drugs due to the certain safety problem of synthetic antioxidant drugs such as liver damage and carcinogenic [2]. Polysaccharide is natural antioxidant drug that have been widely researched.

Sulfated seaweed polysaccharide has biological activities such as anti-coagulation, anti-tumor, anti-inflammatory, antiviral and antioxidant activities [3], and great medicinal value. The physicochemical properties of polysaccharide (such as molecular weight, monosaccharide composition, protein and polyphenol content), especially the -OSO₃H content is the important factor affecting antioxidant activity of polysaccharide. Compounds with structures containing one to two or more groups such as -OSO₃H, -OH, -SH, -COOH, PO₃H₂, C=O, -NR₂, -S, and -O- will have stronger free radical scavenging ability [4]. Li et al [5] studied the antioxidant activities of three acid seaweed polysaccharides which can scavengve DPPH radical, hydroxyl radical and have better chelating capacity and reducing power. Tan et al [6] studied the scavenging activities on DPPH and ABTS radicals of polysaccharide extracted from
Dipsacus asperoides roots (DAP) and found that they have very strong scavenging capacity, suggested that it demonstrated high antioxidant activity and repair the damaged cell induced by H$_2$O$_2$.

Sargassum fusiform is a well-known edible algae, whose polysaccharides have been proved to possess interesting bioactivities like antitumor, antioxidant, antimicrobial, anticoagulant and immunomodulatory activities [7]. The researches showed that the Sargassum fusiform polysaccharide consisted of mannose, glucose, galactose, xylose, fucose and glucuronic acid or galacturonic acid, or both uronic acids [8].

**Experiments**

**Reagents and Apparatus**

Phosphate buffer solution (PBS), 1,1-diphenyl-2-trinitrobenzene hydrazine (DPPH) and other conventional reagents were analytical pure and brought from Guangzhou danielspulber instrument Co., Ltd. Sargassum fusiform polysaccharide (SFP) was produced by Beijing Newprobe Bioscience & Technology Co., Ltd. The content of polysaccharide was equal to or more than 98%. Other conventional reagents were of analytically pure. Table 1 showed the basic physical and chemical properties of rude and degraded sargassum fusiform fractions.

Instrument included UV-Vis spectrophotometer (Cary 500, Varian Company, and USA).

**Degradation of SFP**

SFP was degraded by hydrogen peroxide (H$_2$O$_2$). In brief, SFP (1.2 g) was dissolved in 40 mL distilled water at 70 °C. After heating to 90 °C, H$_2$O$_2$ (27 ml) with the concentration of 1%, 4%, 8%, and 12% were introduced directly into the solution. The degradation reaction was allowed to proceed for 2 h, at that point the pH of solution was adjusted to 7.0 by adding 2 mol/L sodium hydroxide solution. Then, the resultant was precipitated by addition of anhydrous ethanol to the actual volume. The solution was stored at 4 °C overnight and then filtered. The filtrated was washed with anhydrous ethanol twice and dried in a vacuum.

**Determination of Average-molecular-weight (Mw) and Sulfate-group Content**

Average M$_w$ of SFP was determined by Ubbelohde viscosity method. After measuring the falling time in the viscometer, relative ($\eta_r$) and specific ($\eta_{sp}$) viscosity could be calculated from $\eta_r = T/T_0$ and $\eta_{sp} = \eta_r - 1$, where $T_i$ and $T_0$ are the falling time of SFP solutions and deionized water, respectively. Intrinsic viscosity ($\eta$) could be calculated according to the one-point method, i.e., $\eta = (2(\eta_{sp}) - \ln(\eta_{sp}))^{1/2}/c$, in which $c$ is the concentration of samples to be tested. The Mw of SFP was calculated through its value $\eta$. The relationship between $\eta$ and the Mw M of the polymer solutions could be described by the Mark–Houwink empirical equation $\eta = \kappa M^\alpha$, where $\kappa$ and $\alpha$ were constants related to polymer nature, solvent, and temperature. The sulfate-group (–OSO$_3$H) content of SFP was determined by the BaCl$_2$-gelatin turbidity method.

**Hydroxyl Radical (•OH) Scavenging Activity of Polysaccharide**

The •OH scavenging ability of polysaccharide in vitro were detected by H$_2$O$_2$/Fe system method. The reaction mixture that contained different concentrations of polysaccharides (0.15–3.0 mg/mL, 1 mL), was incubated with phenanthroline (2.5 mmol/L, 1 mL), ferrous sulfate (2.5 mmol/L, 1 mL) and H$_2$O$_2$ (20 mmol/L, 1 mL) in phosphate buffer (20 mmol/L, 1 mL, pH 7.4) for 90 min at 37 °C. The absorbance was measured at 536 nm and the average
absorbance was $A_3$. The absorbance when both hydrogen peroxide ($H_2O_2$) and polysaccharide solution or polysaccharide solution alone was replaced with distilled water was $A_2$ and $A_1$, respectively. The ascorbic acid ($Vc$) was used as positive control group. The ability to scavenge hydroxyl radicals was calculated using the following equation:

$$\text{Scavenging effect (\%)} = \frac{(A_3 - A_1)}{(A_2 - A_1)} \times 100\%$$

**DPPH Radical Scavenging Activity of Polysaccharide**

The DPPH radical scavenging activity was carried out according to Wang et al [9] with minor modification. The absorbance was measured at 517 nm. The $Vc$ was used as positive control group. The ability to scavenge DPPH radicals was calculated using the following equation:

$$\text{Scavenging effect (\%)} = \left[1 - \frac{(A_2 - A_1)}{A_0}\right] \times 100\%$$

where $A_2$ was the absorbance of 3 mL sample mixed with 1 mL DPPH solution; $A_1$ was the absorbance of 3 mL sample mixed with 1 mL blank solvent (dehydrated alcohol); $A_0$ was 1 mL DPPH solution mixed with 3 mL blank solvent.

**ABTS Radical Scavenging Activity of SFP**

7 mmol/L ABTS solution mixed with 2.45 mmol/L potassium persulfate aqueous solution and leaving the mixture in the dark at room temperature for 12-16 h. Then 3.0 mL mixture solution was added to 1 mL of various polysaccharide solution (0.15-3 mg/mL) in test tube. After reacting for 6 min at room temperature, the absorbance was measured at 734 nm.

$$\text{Scavenging effect (\%)} = \left[1 - \frac{(A_1 - A_2)}{A_0}\right] \times 100\%$$

where $A_0$: control group without polysaccharide; $A_1$: experiment group; $A_2$: without reagents (the absorbance of polysaccharides solution ($A_2$) was 0).

**Reducing Power of Polysaccharide**

2.5 mL of samples in different concentrations (0.15–3.0 mg/mL) was mixed with 2.5 mL phosphate buffer (PBS, pH 6.6) and 2.5 mL potassium ferricyanide (1.0%, w/v). The mixture was incubated at 50 °C for 30 min and cooled to room temperature. 2.5 milliliter of TCA (10%, w/v) was added to the mixture which was then centrifuged for 10 min at 3000 r/min. The supernatant (2.5 mL) was mixed with 0.5 mL $FeCl_3.6H_2O$ (0.1%, w/v) solution and 5.0 mL distilled water. The mixture was fully mixed and stood for 10 min. The absorbance was measured at 700 nm. The ascorbic acid ($Vc$) was used as positive control group and for comparison.

**Ferrous ion Chelating Ability of SFP**

1.0 mL polysaccharide solution in different concentrations were mixed with ferrous chloride (0.05 mL, 2.0 mmol/L), 0.2 mL ferrozine (5.0 mmol/L) and 2.25 mL distilled water. After mixing for 10 min, ferrozine can form stable complexes with $Fe^{2+}$, which has characteristic absorption at 562 nm and can be detected by UV-Vis spectrophotometer. When the polysaccharide solution was added, $Fe^{2+}$ content was decreased due to the chelation of polysaccharide and $Fe^{2+}$, and the absorbance was reduced. In the blank group, polysaccharide solution was substituted with distilled water. In the control group, ferrous chloride solution was substituted with distilled water. EDTA was used as positive control. The ion-chelating activity of polysaccharides was calculated as:

$$I/\% = \frac{[A_0-(A_1-A_2)]}{A_0} \times 100\%,$$

where $A_1$, $A_2$ and $A_0$ were the absorbances of the sample, control group and blank group, respectively.
Table 1. Degraded conditions of four sargassum fusiform polysaccharide (SFP) fractions with different molecular weights and their physical and chemical properties.

<table>
<thead>
<tr>
<th>SFP abbreviation</th>
<th>Concentration of H₂O₂ / %</th>
<th>Mean molecular weights M_r / kDa</th>
<th>–OSO₃H content /%</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFP</td>
<td>0</td>
<td>16.2</td>
<td>8.36</td>
</tr>
<tr>
<td>SFP1</td>
<td>4</td>
<td>8.21</td>
<td>8.51</td>
</tr>
<tr>
<td>SFP2</td>
<td>8</td>
<td>3.83</td>
<td>8.54</td>
</tr>
<tr>
<td>SFP3</td>
<td>12</td>
<td>2.23</td>
<td>8.62</td>
</tr>
</tbody>
</table>

[*] Degradation T: 90°C; degradation time: 2 h.

Results and Discussion

Hydroxyl Radical (•OH) Scavenging Capacity of SFPs with Different Molecular Weights

•OH is a radical harmful to organisms. Polysaccharide can supply a single electron or hydrogen atom, which combine with •OH to achieve radical scavenging activity. Fig. 1A shows the •OH scavenging capacity of four fractions of SFP, their molecular weight was 2.23, 3.83, 8.21, and 16.2 kDa, respectively. The findings are as follows:

1) The hydroxyl radical scavenging capacity increased with increasing concentration of each polysaccharide, indicating the concentration-dependent antioxidant activity of the polysaccharides.

2) The hydroxyl radical scavenging capacity gradually increased as the molecular weight of SFP decreased from 16.2 kDa to 2.23 kDa.

![Figure 1](A) ![Figure 1](B)

Figure 1. Concentration effect of hydroxyl radical (A) and DPPH radical (B) scavenging rate of four fractions of SFP. Values are means±S.D. (n=3).

Comparison of DPPH Radical Scavenging Capacity

DPPH is a stable free radical widely used to determine the free radical-scavenging activities of antioxidants. DPPH free radical shows a characteristic absorption at 517 nm (purple); and when it is reduced by antioxidants to the non-radical form DPPH-H, the purple color fades rapidly [9]. Fig. 1B shows the DPPH radical scavenging activities of four SFP fractions with different molecular weights. All the polysaccharide fractions exhibited concentration-dependent DPPH radical scavenging capacity.
The DPPH radical scavenging capacity gradually increased as the molecular weight of SFP decreased from 16.2 kDa to 2.23 kDa. This finding is consistent with the rule of hydroxyl radical scavenging shown in Fig. 1A.

**Comparison of ABTS Radical Scavenging Capacity**

ABTS assay is an important method used to measure the antioxidant capacity of antioxidants. Fig. 2A shows the ABTS radical scavenging activities of the four SFP fragments with different molecular weights. All the polysaccharides exhibited the ABTS radical scavenging capacity in a concentration-dependent manner; high concentrations of polysaccharide indicated strong scavenging capacity.

The ABTS radical scavenging capacity gradually increased with decreasing molecular weight of SFP; the maximum inhibition was detected in the fraction with a molecular weight of 2.23 kDa. In general, the ABTS radical scavenging capacity of antioxidants was positively associated with DPPH radical scavenging capacity [10].

**Comparison of Reducing Power**

The antioxidant ability of polysaccharides is positively to its reducing power, and strong reducing power indicates strong antioxidant ability. Natural antioxidants can terminate free radical chain reaction by donating an electron or hydrogen atom to the free radical. Thus, reducing power is an important index of potential antioxidant capacity of antioxidants.

As shown in Fig. 2B, the absorbance measured at 700 nm increased with the increasing concentration of polysaccharide, suggesting that the reducing power was strengthened in a concentration-dependent manner.

For the different polysaccharides, the absorbance, that is, the reducing power of polysaccharide, increased with the decreasing the molecular weight.

![Figure 2](image)

Figure 2. Concentration effect of ABTS radical scavenging rate (A) and reducing power (B) of four fractions of SFPs. Values are means±S.D. (n=3).

**Comparison of Chelating Effect on Ferrous Ions**

Metal chelating ability might be involved in antioxidant activity and affects other functions that contribute to the antioxidant activity. Ferrous ions (Fe^{2+}) are the most effective antioxidants in the food system, Fe^{2+} can stimulate lipid peroxidation and accelerate the oxidation of lipid compounds through the Fenton reaction. Therefore, the chelating effect of
polysaccharides on ferrous ions might affect the other activities of scavenging free radicals to protect organisms against oxidative damages [11].

As shown in Fig. 3, all SFP fractions showed Fe$^{2+}$ chelating capacity in a concentration-dependent manner. The chelating ability of ferrous ions strengthened with decreasing molecular weight of polysaccharides at the same concentration. The active groups (such as -SO$_3$H and -COOH) of polysaccharides with low molecular weight was exposed and possessed high degree of freedom; therefore, the space steric of Fe$^{2+}$ chelating was small and the chelating capacity increased.

![Chelating ability/ %](chart.png)

Figure 3. Relationship between ferrous ions chelating ability and concentrations of SFP with different molecular weight. Values are means±S.D. (n=3).

Conclusions

Original SFP with a molecular weight of 16.2 kDa was degraded to obtain three fragments with low molecular weights of 8.21, 3.83, and 2.23 kDa. All polysaccharides exhibited scavenging activity on hydroxyl, DPPH, and ABTS radicals and reducing power. Also the Fe$^{2+}$ chelating ability increased with decreasing molecular weight of polysaccharides. That is, SFP3, with the lowest molecular weight, exhibited the strongest antioxidant activity. The degraded polysaccharide fractions, especially SFP3, may be potential anti-stone drugs.

Acknowledgements

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References


