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Hypolipidemic and antioxidant properties of a polysaccharide fraction from *Enteromorpha prolifera*

Zhihong Tang[a], Hongwei Gao[b], Shuai Wang[a], Shaohong Wen[a], Song Qin[c,*]

[a] College of Life Science, Yantai University, Yantai, Shandong Province 264005, PR China
[b] Beijing Institute of Transfusion Medicine, Beijing 100850, PR China
[c] Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Shandong Province 264003, PR China

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ABSTRACT

A polysaccharide fraction (EPF2) was obtained from the crude polysaccharides of *Enteromorpha prolifera* by a series isolation procedure. Monosaccharide components analysis indicated that EPF2 was composed of rhamnose, xylose, mannose, galactose and glucose in a molar ratio of 3.64:1.08:0.21:0.75:0.27. Hypolipidemic and antioxidant properties of EPF2 were investigated. The results showed that the hypolipidemic effect of EPF2 was in a concentration-dependent fashion and the prior oral administration of EPF2 (300 mg/kg body weight) exhibited considerable effect which could bear comparison with that of simvastatin. Moreover, administration of EPF2 could significantly enhance the activities of endogenous antioxidant enzymes and lowered the content of maleic dialdehyde (MDA) in serum. The results suggested that EPF2 had a high hypolipidemic activity and this activity might be attributed to its antioxidant potential.

1. Introduction

Enteromorpha prolifera, distributed widely in the intertidal zone of the ocean, is one of the most common green algae. In ancient times, *E. prolifera* was not only used as food, but was also used as a traditional herb for the treatment of many diseases, such as dissipating heat and treating hydropic diseases, *etc.*, as documented in Chinese materia medica. *E. prolifera* polysaccharides (EP), one of the main active ingredients of *E. prolifera*, are attributed to many healing properties of the herb. Recently, it has been reported that EP have many functions such as immunity regulation, antimicrobial, anti-virus, antitumor, and hypolipidemic [1–6]. Though previous studies have shown that crude polysaccharides from *E. prolifera* can reduce blood lipid [5,6], whether its purified fraction possesses hypolipidemic effect has not been evaluated. In our earlier research [7], antioxidant activity tests *in vitro* systems revealed the polysaccharides from *E. prolifera* possessed considerable antioxidant activity. However, it is unclear whether they also show antioxidant activity *in vivo*. In this study, a polysaccharide fraction EPF2 was obtained from *E. prolifera* by a series isolation procedure. In addition, hypolipidemic and antioxidant properties of EPF2 in mice fed high-fat diet were investigated.

2. Materials and methods

2.1. Materials

E. prolifera was collected in the coast of Qingdao in China. Kunming mice were purchased from Experimental Animal Center of Shandong University. L-rhamnose, D-glucuronic, D-arabinose, D-xylose, D-fructose, D-galactose and D-mannose were from Sigma Chemical Co. (St. Louis, MO, USA), while DEAE-Sepharose CL-6B and Sephadex G-200 were purchased from Amersham Biosciences Co. (Uppsala, Sweden). Reagents for the detection of TC, TG and HDL-c were obtained from Beijing Chengxinde Biochemistry Reagent Company (Beijing, PR China). Assay kits for malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) were the products of NanJing Biotechnology Co. Ltd. (NanJing, China). Other reagents were of analytical grade.

2.2. Isolation and purification of polysaccharides

EPF2 was prepared from *E. prolifera* according to our previous method [7] with some modification. Dried *E. prolifera* (1000 g) was ground in a blender to obtain a fine powder and treated with 80% ethanol at 75 °C for 6 h to remove some small molecule materials. The pretreated sample was extracted by water at 90 °C for 3 h. The water extraction solutions were centrifuged (4000 × *g*, 10 min), and then the supernatant was deproteinated according to Sevag method [8]. The resulting aqueous fractions were extensively

* Corresponding author. Tel.: +86 5352109018; fax: +86 5352109018.
E-mail addresses: sqin@yic.ac.cn, qins607@yahoo.cn (S. Qin).

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dialyzed against running distilled water for 2 days. The retentate was precipitated by the addition of ethanol to a final concentration of 75% (v/v), and the precipitates were collected by centrifugation (4000 × *g*, 10 min), then solubilized in deionized water and lyophilized to get the crude polysaccharides, the yield was 7.9%.

The crude polysaccharides (1000 mg) were dissolved in 15 mL distilled water, centrifuged, and then the supernatant was applied to a column (40 cm × 5.5 cm) of DEAE-Sepharose CL-6B equilibrated with distilled water. After loading with sample, the column was eluted with distilled water, followed by stepwise elution with increased concentration of NaCl (0.2, 1.0 and 1.5 M), respectively, at 4 mL/5 min/tube. The major polysaccharide fraction was collected at 1.0 M NaCl, dialyzed against tap water and distilled water for 48 h, then purified by gel-fltration chromatography on a column of Sephadex G-200 (60 cm × 2.8 cm), and finally lyophilized to get the polysaccharide fraction named EPF2.

2.3. Characterizations of EPF2

The carbohydrate content was examined by phenol–sulfuric acid colorimetric method using glucose as a standard [9]. Protein content was measured according to Bradford's method, using bovine serum albumin (BSA) as the standard [10]. Sulfate group was determined according to the reported methods [11]. The uronic acid content was examined by 3-hydroxydiphenyl assay using glucuronic acid standard [12].

The monosaccharide analysis was performed by gas chromatography (GC) method as described [13] on a HP5890 instrument (Hewlett-Packard Component, USA) with a column HP-5 (30 m × 0.32 mm × 0.25 μm).

Homogeneity and average molecular weight of were determined by HPGPC method on a Waters HPLC apparatus (Waters 515, Waters Co. Ltd., USA) equipped with an Ultrahydrogel^{TM}2000 analytical column eluted with double distilled water at the flow rate of 1.0 mL/min. The injection concentration and volume of the sample were 2 mg/mL and 15 μL respectively. The molecular weight was measured, relative to Dextran standards (T-10, T-40, T-70, T-500 and T-2000), according to the method by Wang et al. [14].

The Fourier-transform infrared (FTIR) spectrum of the sample was carried out by the potassium bromide (KBr) pellet method [15] on a Fourier-transform infrared spectrophotometer (Nexus 670 FTIR, Thermo Nicolet, USA) in the frequency range of 400–4000 cm^{-1}.

2.4. Animal grouping and experimental design

The assay of effect of EPF2 on blood lipid and oxidative stress in high-fat diet-induced mice fed high-fat diet was carried out according to the reported method [16] with some modifications. Sixty male kunming mice, body weight 20 ± 2 g, were maintained under controlled conditions of 12 h light/12 h dark cycle and 50–60% relative humidity at 25–30 °C. After adapting to their environment for 1 week, these mice were randomly divided into six groups (ten animals for each) for experiment. One group was the control group (I). The others were experimental groups: HF group (II), simvastatin group (III) and three EPF2 treated groups (IV, V and VI). Mice in Group I were allowed free access to standard feed, water and orally administrated with the same volume of physiological saline for 30 days. Mice in Group II were allowed free access to high-fat feed, water and treated with the same volume of physiological saline for that same period. Mice in Group III were allowed free access to high-fat feed, water and treated by oral infusion with simvastatin at a dose of 4 mg/kg body weight dissolved in physiological saline for that same period. Mice in Group IV, V and VI were allowed free access to high-fat feed, water and were treated by oral infusion with EPF2 dissolved in physiological saline daily for that same period, at a dosage of 100, 200 and 300 mg/kg body weight, respectively. The doses used in this study were confirmed to be suitable and effective in tested mice according to preliminary experiments. High-fat diet consists of 84% basic diet, 5% lard, 1% cholesterol and 10% surcrose.

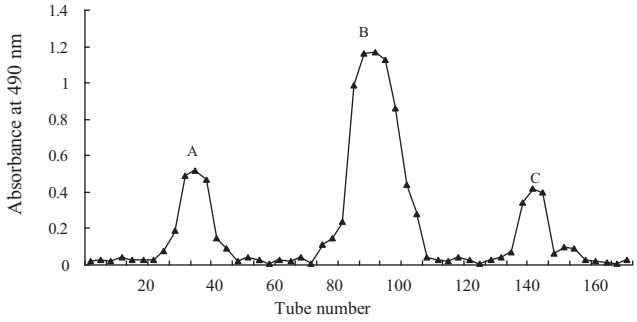

Fig. 1. DEAE-Sepharose CL-6B column chromatography of crude polysaccharides of *Enteromorpha prolifera*. (A) Eluted with 0.2 M NaCl; (B) eluted with 1.0 M NaCl; (C) eluted with 1.5 M NaCl.

2.5. Biochemical assay

Twenty-four hours later after the last drug administration, the mice were killed by cervical dislocation. Blood samples were collected and centrifuged (1500 × *g*, 10 min) at 4 °C to obtain serum. The serum TC, TG and HDL-c concentrations were determined using an enzymatic method (Sigma Diagnostics, St Louis, MO). The serum concentration of LDL-c was estimated using Friedewald's method [17]. The activities of SOD, CAT, GSH-Px and level of MDA were measured by using commercial reagent kits, according to the instruction manuals. In brief, enzymatic activities of SOD, CAT and GSH-Px were determined according to the methods of xanthine oxidase–xanthine reaction system, CAT-H_2O_2 reaction system and reduced glutathione (GSH)-H_2O_2 reaction, respectively. All above enzymatic activities were expressed as unit per milliliter in serum (U/mL). MDA level was measured by using 2-thiobarbituric acid (TBA) method and expressed as nanomole per milligram of protein (nmol/mg protein) in liver or nanomole per milliliter in serum (nmol/mL).

2.6. Statistics

All data were given as means ± standard deviation (SD). Analysis of variance (ANOVA) was performed for the effect of comparisons between the means of various treatment groups and the paired Student's *t*-test was used to determine significant differences among groups. All analysis were performed using Statistical Package for Social Sciences (SPSS) version 12.0 and the level of significance was accepted at $P < 0.05$.

3. Results

3.1. Characterization of EPF2

The crude polysaccharides from *E. prolifera* were obtained as water-soluble dust-colored powder. Then crude polysaccharides were further isolated through DEAE-Sepharose CL-6B column (Fig. 1). The major polysaccharide fraction was collected at 1.0 M NaCl elute, then purified through Sephadex G-200 column, and finally lyophilized to get the polysaccharide fraction named EPF2. The carbohydrate content, protein content, uronic acid content and sulfate content of EPF2 were determined. The results showed that EPF2 contained 53.2% carbohydrates, 11.5% proteins, 18.6% sulfate group and 12.4% uronic acid. Monosaccharide components analysis revealed that EPF2 was composed of rhamnose, xylose, mannose,