Protective effect of polysaccharides from Sargassum fusiforme against UVB-induced oxidative stress in HaCaT human keratinocytes

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In this study, Sargassum fusiforme polysaccharide was purified using a DEAE-Sepharose fast-flow column to obtain Sargassum fusiforme polysaccharides fraction (SFP-P1). As expected, the SFP-P1 contains mainly carbohydrates (87%) with less than 1% protein. Within the carbohydrate, it contains about 10% sulfate and 18% uronic acids. SFP-P1 consisted of D-fucose (13.17%), galactose (4.28%), glucose (1.95%), xylose (5.50%), and mannose (75.10%) with an average molecular weight of 113 kDa. Mannose and D-fucose were two predominant monosaccharides. In addition, (1 → 3)-linked or (1 → 6)-linked, (1 → 2)-linked or (1 → 4)-linked and (1 → 3)-linked glycosyl linkages accounted for 1.62%, 60.69% and 37.69% of all linkages in the molecule, respectively. The glycosidic linkage types of SFP-P1 were proven to be (3,6)-α-D-Manp (1 →, → 4)-β-α-D-GalAp (1 →, → 4)-β-α-D-Xylp (1 →, → 3,4)-α-β-GlcAp (1 →). The results indicated the SFP-P1 had cytoprotective activity against UVB-induced oxidative stress in HaCaT cells through stimulation of SOD, GSH-Px enzymes activities and ROS. Also SFP-P1 suppressed the UVB-induced expression of MMP-1 & MMP-9.

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1. Introduction

Human skin, as the largest and visible organ of the body, which can be divided into three layers including epidermis, dermis and hypodermis (Hou et al., 2012). Skin is an effective physical and chemical barrier against the oxidative environment directly including solar ultraviolet (UV). UV can be divided into UVA (320–400 nm), UVB (290–320 nm) and UVC (100–290 nm) based on wavelength. Among these, UVC is absorbed by the ozone layer which blocked from reaching the earth’s surface (Dai et al., 2011). UV irradiation is mainly composed of UVA and UVB rays. Though UVB is a minor part of solar UV irradiation, which is 1000 times more capable of photoaging damage than UVA (Martinez et al., 2015; Matsumura & Ananthaswamy, 2004). Excessive exposure of the skin to UVB irradiation causes several pathological changes which are characterized by erythema, edema, sunburn, hyperplasia, inflammation, immune suppression, photoaging, and carcinogenesis (Martinez et al., 2015). Chronic exposure of the skin to UVB radiation could increase the generation of excessive reactive oxygen species (ROS) and decrease antioxidant levels in the skin (Fan, Zhuang, & Li, 2013). Excessive amount of ROS would break the balance of antioxidant defense systems which directly increase oxidative DNA damage and peroxidation of lipid and protein in the skin, and finally cause oxidative stress and photoaging (Lee, Ko et al., 2013; Lee, Yang et al., 2013). UVB-induced ROS mediated the epidermal growth factor and cytokines binding to the receptors which would activate the matrix metalloproteinases (MMPs) in both the epidermis and dermis (Tiraravesit et al., 2015), and then promote collagen breakdown by upregulating extracellular matrix and elastin in the dermis (Lee, Ko et al., 2013; Lee, Yang et al., 2013). Collagen, the most abundant extracellular matrix (ECM) proteins which are the main building blocks of the skin in the dermis, is derived from dermal fibroblasts and regulated by mitogen-activated protein (MAP) kinase (Halliday, 2005). MAP kinase induces activator protein-1 (AP-1; a transcription factor containing c-Fos and c-Jun) and promotes collagen breakdown by upregulating enzyme called matrix metalloproteinases (MMPs). MMPs are a family of zinc dependent enzyme related matrix-degrading which played crucial roles in skin aging and photodamage. Especially, MMP-1, known as an interstitial collagenase, is the major collagen-degrading enzyme which is related to the degradation of collagen type I, II and III in the skin (Chiang, Chan, Chu, & Wen, 2015). In addition, MMP-9 also plays vital roles in photoaging by degrading ECM in the dermis (Chiang et al., 2015). More and...
more scholars pay attention to the skin care for maintenance of healthy skin in recent years. Various botanical antioxidants supplement indicated protective effects against UVB-induced skin injury, such as chlorogenic acid (Cha et al., 2014), phenolic (Kim, Park, Lee, Lim, & Nho, 2015), flavonoids (Chiang et al., 2015) and collagen peptide (Fujii et al., 2013). However, little work has been done on the skin protective effect of polysaccharides from Sargassum fusiforme. Sargassum fusiforme, belonging to the family of Sargassaceae, is a kind of brown algae extensively distributed in the coastal zones in Asia (Cong et al., 2016). Sargassum fusiforme not only has been used as a Chinese health food but also as herbal medicine for thousands of years (Hu et al., 2016). Sargassum fusiforme contains many nutritional components, such as polysaccharides, protein, minerals, vitamins and dietary fiber (Zhou, Hu, Wu, Pan, & Sun, 2008). Modern pharmacological studies demonstrated that Sargassum fusiforme polysaccharides possessed multiple functions such as antitumor (Chen et al., 2012), antioxidant (Wang et al., 2013), hypolipidemic, immunomodulatory activities (Chen et al., 2012; Hu et al., 2014). Due to these multiple biological activities, structures and properties of Sargassum fusiforme polysaccharides (SFP) have been investigated widely. However, little work has been reported on the relationships between key structural characteristics of SFP and its biological activity. Therefore, in order to explore and utilize Sargassum fusiforme resource, it is necessary to investigate the structural properties of Sargassum fusiforme polysaccharides and their biological activities. In this study, Sargassum fusiforme polysaccharides were extracted by hot water and purified using a DEAE-Sepharose fast-flow column to obtain SFP-P1 fraction. The chemical composition, preliminary structural properties and protective effects against UVB-induced oxidative stress of SFP-P1 were investigated.

2. Experimental

2.1. Chemicals and reagents

Sargassum fusiforme was collected from Dongtou (Zhejiang China) in May 2015. DMEM was purchased from Gibco Biotechnology Co. (Grand Island, NY, USA). The human keratinocyte cells were obtained from Jennio Biological (Guangzhou, Guangdong, China). The assay kits for superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and reactive oxygen species (ROS) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China). MMP-1 and MMP-9 ELISA assay kits were purchased from Neobiocine Technology Company (Shenzhen, Guangdong, China). All other chemicals used were analytical grade purchased from Guangzhou Reagent Co. (Guangzhou, Guangdong, China).

2.2. Preparation of crude polysaccharides

Sargassum fusiforme polysaccharide was extracted by hot-water and ethanol precipitation as previous report (Wen et al., 2016). Dried Sargassum fusiforme powder was refluxed with four volumes of 95% ethanol at 70 °C for 4 h to de-color and remove ethanol-soluble substances. Then the mixture was filtrated by Whatman No. 1 filter paper, and the residue was collected and dried at 50 °C for 12 h. These residues were extracted with boiling water at a ratio of 50:1 (v/w) for 4 h. The supernatants were centrifuged (Allegra X-15R, Beckman Coulter Co., Ltd., Brea, CA, USA) at 3500g for 10 min, and then the extracts were filtrated by Whatman No. 1 filter paper. The water-extracts was concentrated to 1/4 volume under reduced pressure at 55 °C using a rotary evaporator (Hei-VAP, Heidolph, Germany), and precipitated with anhydrous ethanol (1:4, v/v) kept for overnight at 4 °C. The precipitates were obtained by centrifugation at 3500g for 15 min and deproteinized using the Sevag method (Staub, 1965). Then the Sargassum fusiforme polysaccharides (SFP) was obtained after the precipitates, freeze-dried and stored in a desiccator at room temperature.

2.3. Separation and purification of SFP

Crude Sargassum fusiforme polysaccharides (160 mg) dissolved in 3 mL of distilled water was applied to the DEAE-Sepharose fast-flow column (26 mm × 20 cm) which was eluted with 200 mL of distilled water 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 M NaCl at a flow rate of 1 mL/min. Each fraction of 5 mL eluent was collected with an automatic collector (BSZ-160, Shanghai Precision Scientific Instrument Co., Ltd, Shanghai, China) and detected by the phenol-sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) to obtain the elution curve (Fig. 1) at 490 nm.

2.4. Determination of chemical composition

The carbohydrate content of SFP-P1 was measured by the phenol-sulfuric acid method with d-glucose as the standard (Dubois et al., 1956). The protein content of SFP-P1 was measured by Lowry method with BSA as the standard (Lowry, Rosebrough, Farr, & Randall, 1951). The uronic acid content of SFP-P1 was measured by the carbazole-sulfuric acid method with glucuronic acid as standard (Li, You et al., 2015; Li, Huang et al., 2015). The sulfate content of SFP-P1 was measured by the barium chloride-gelatin nephelometry method (Zhang et al., 2015).

2.5. Determination of molecular weight

The molecular weight determination of SFP-P1 was determined by high-performance gel permeation chromatography (HPGPC) as described previously (You et al., 2013). The TSK-GEL columns (Tosoh Co., Ltd, Tokyo, Japan) were in the series of TSK-GEL guard column (6.0 × 40 mm), G-4000 PWXL (7.8 × 300 mm) and G-2500 PWXL (7.8 × 300 mm). These were eluted with 0.02 M KH2PO4 (pH 6.0) at a flow rate of 0.6 mL/min. The column temperature was maintained at 35 ± 0.1 °C. The injection volume was 20 μL in each run. The molecular weight of SFP was measured based on the equation of elution volume being proportional to the logarithm of molecular weight of standard dextrins.

![Fig. 1. DEAE-Sepharose fast-flow chromatogram of the crude Sargassum fusiforme polysaccharides.](image-url)
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