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The effect of different substitute groups and molecular weights of fucoidan on neuroprotective and anticomplement activity

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ABSTRACT

Ten fucoidan (FPS) derivatives were successfully synthesized, and their potential neuroprotective and anticomplement activities were investigated employing various established *in vitro* systems. The aim of the present study was to investigate the effects of different substitute groups and molecular weights of fucoidan on neuroprotective and anticomplement activities. All FPS derivatives possessed considerable neuroprotective and anticomplement activities and had stronger activities than FPS in certain tests. The *in vitro* results found that sulfated and benzoylated derivatives could reverse the decreased mitochondrial activity and decreased lactate dehydrogenase (LDH) and reactive oxygen species (ROS) release induced by 6-hydroxydopamine (6-OHDA, $P < 0.01$ or $P < 0.001$), which provides further evidence that sulfate and benzoylate groups could enhance the neuroprotective activity of fucoidan. In anticomplement experiments, all samples showed anticomplement activity in both systems; however, the sulfated and benzoylated derivatives showed better activity than fucoidan, with higher molecular weights showing the strongest activity. Available data suggested that substituted groups of fucoidan played an important role on neuroprotective and anticomplement activities. The mechanism of the influence of the neuroprotective and anticomplement activities of samples of the substituted groups was indicated.

1. Introduction

Fucoidans are sulfated polysaccharides extracted from brown seaweeds and some invertebrates, such as sea cucumber and sea urchin. The chemical composition and structure of fucoidans are complicated, and these features vary depending on different sources and extraction methods. Fucoidans exhibit excellent activities, such as antioxidant, antitumor, antithrombosis, and neuroprotection; thus, several research groups have examined these molecules over the last thirty years. Increasing evidence shows that the activity of fucoidans is associated with their chemical composition and structure. Molecular weight (Mw) is an important factor affecting fucoidan activity. The relationships between the Mws of fucoidans and their antioxidant activities are not simply linear. Samples with Mws of 3.8, 1.0, and >8.3 kDa have better hydroxyl radical scavenging activity, reducing power and superoxide anion scavenging activity, respectively [1]. Molecular weight also affects anticoagulant activity. The binding of fucans with almost the same sulfate content to fibrinogen changed with different molecular weights [2,3]. The other factors include different substitute groups and the linkage position. Key sulfated groups exhibit and maintain fucoidan activity. Oversulfated fucoidans exhibited better superoxide radical scavenging activity compared with native fucoidans [4]. Other substitute groups, such as acetylated, benzoylated, phosphorylated and aminated groups, exhibited different effects in certain experiments. Acetylated and phosphorylated substitute groups could enhance the antioxidant activity of fucoidan in scavenging DPPH radical and reducing power experiments [5]. The *in vitro* anticoagulant activity of fucoidan and its derivatives were tested using activated partial thromboplastin time (APTT), prothrombin time (PT), and thrombin time (TT) assays. The results showed that all samples had anticoagulant activity in APTT and TT assays, and only fucoidan derivatives affected the PT assay. Among all samples, aminated fucoidan showed the strongest anticoagulant activity. We proposed that amino groups have positive charges and can change the charge density of fucoidans [2]. Benzoylated substitute groups could enhance renal protective activity. The elevated serum urea nitrogen (SUN) and serum creatinine (SCr) levels significantly decreased with benzoylated fucoidan administration. The underlying mechanism is that benzoylated fucoidan could enhance the activity of antioxidant enzymes and reduce lipid peroxidation (LPO) levels, which could alleviate the symptoms of Chronic renal failure (CRF) [6].

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From these results, we propose that more active samples could be identified in certain systems through making fucoidan derivatives with different molecular weights and substitute groups.

Parkinson's disease (PD) is a common disease in elderly individuals, the ratio of the PD patient is increasing yearly; thus it is necessary to identify effective and safe drugs. The mechanism of PD is complicated, as dopaminergic neuron loss is a primary feature in the brains of PD patients [7]. However, when a patient shows PD symptoms, nearly 70% of their dopaminergic neurons in the brain have already been lost. Clinical drugs primarily delay/prevent PD patients from developing a more serious stage of disease [8]. However, the disease was difficult to completely cure. The *in vitro* and *in vivo* experiments showed that compounds that can protect against dopaminergic neuron death can also alleviate other symptoms of PD and delay the development of this disease [9]. Increasing studies have focused on finding neuroprotective drugs to prevent dopaminergic neuron loss in the early stages of PD and regenerate dopaminergic neurons [10]. In a previous study, we observed that fucoidan had neuroprotective activity and the mechanism was associated with its antioxidant and antiapoptotic activities. We also found that different fucoidan fractions with different monosaccharide compositions and different sulfate group contents exhibited a variety of neuroprotective activity. Samples with more uronic acid and complicated monosaccharides with less sulfate groups showed the highest neuroprotective activity [11]. However, how the different substitute groups of fucoidan influence neuroprotective activity is still unclear. In the present study, we synthesized ten fucoidan derivatives with different substitutes on two molecular weight levels, and tested their neuroprotective activity *in vitro*.

The complement system is one of the important immune defense systems in the human body. This system plays an important role in the elimination of foreign microorganisms and the maintenance of the balance of the body. Inhibiting the excessive activation of the complement system has some preventive and therapeutic effects on many diseases, such as systemic lupus erythematosus, rheumatoid arthritis, acute respiratory distress syndrome, *etc.* [12,13]. Currently, there is no ideal therapeutic drug for this kind of disease. Therefore, a new type of complement inhibitor with high efficiency, low toxicity and high specificity is urgently needed in the clinic. Polysaccharides are an efficient type of complement inhibitor. Wang et al. reported two homogeneous polysaccharides from *Eclipta prostrata* that exhibit anti-complement activity, as preliminary mechanistic studies indicated that both polysaccharides inhibited the activation of the complement system by interacting with C1q, C1r, C1s, C2, C4, C5, C7 and C9 components [14]. Two sulfated derivatives of homogalacturonans obtained from preinfused green tea demonstrated a stronger inhibitory effect on complement activation through a classic pathway compared to that of heparin [15]. Zhang et al. found fucoidan extracted from *Kjellmaniella crassifolia* had excellent anticomplement activity in classic and alternative pathways, and also concluded that the molecular weight and content of the sulfate group influenced the anticomplement activity, as fucoidans with low molecular weights and/or less sulfate groups had less or no anticomplement activity [16]. Jin et al. studied the monthly variations of polysaccharides from *Sargassum thunbergii* and their anticomplement activity. The results found that the total sugar and the molar ratio of monosaccharides were different with different harvesting times; however, these differences did not influence the anticomplement activities [17]. However, how different substitute groups of fucoidan influence anticomplement activity was still unclear. The aim of the present study was to demonstrate how the different substitute groups and molecular weights of fucoidan influence its anticomplement activity.

In the present study, we synthesized ten fucoidan derivatives with different substitute groups on two molecular weight levels, and tested their neuroprotective and anticomplement activities *in vitro*. The aim of the present study was to find additional active fucoidan derivatives, which might be used in PD and complement system diseases and could explain the mechanism of different substitute groups and molecular weights in neuroprotective and anticomplement activities.

2. Materials and methods

2.1. Materials

Saccharina japonica (Laminariaceae), cultured in Rongcheng, Weihai, China, was collected in August 2016. The fresh seaweed was immediately washed, sun dried and maintained at room temperature until further use. Monosaccharide standards and dextran molecular weight standards were purchased from Sigma Chemicals Co. All other chemicals and reagents, unless otherwise specified, were not purified, dried or pretreated.

2.2. Extraction and degradation

Fucoidan was prepared as previously described [18]. Low molecular weight fucoidan (DFPS) was prepared according to a previous method used in our laboratory. Briefly, fucoidan was dissolved in water to generate a 2% solution, and then ascorbic acid and hydrogen peroxide were added at concentrations up to 30 mm (30 mmol/L, 1:1). The mixture was stirred for 2 h at room temperature and then the reaction was stopped. The reaction concentrated, dialyzed against tap water for 24 h and distilled water for 24 h using 3600 Da Mw cutoff dialysis membranes. The resultant was concentrated and lyophilized to generate DFPS [3].

2.3. Sulfation of FPS and DFPS [19]

The sulfation reagent, SO_3-DMF, was obtained by dropping 50.0 mL of chlorosulfonic acid into 300 mL of *N*, *N*-dimethylformamide (DMF) under cooling in an ice-water bath. Dry FPS 2 g was added to 80 mL formamide (FA) in three 250-mL bottles, and ten stirred at 50 °C for 30 min. A total of 15 mL of sulfation reagent SO_3-DMF was added dropwise into the FA solution and the reaction was incubated for 3 h at 50 °C. The reaction was then cooled to room temperature, followed by ethanol precipitation. The precipitate was dried, dissolved in distilled water, and the pH was adjusted to 7 using 2 M NaOH. Then, the mixture was dialyzed against tap water for 24 h, followed by distilled water by using 3600 Da Mw cutoff dialysis membranes. The resulting mixture was concentrated and lyophilized to yield oversulfated FPS (SF).

DFPS was sulfated according to the conditions described above and was labeled as SDF.

2.4. Acetylation and benzoylation of fucoidan [4]

Dry FPS 2 g was dissolved in 80 mL formamide, the mixture was stirred at 80 °C for 30 min, and then 50 mL of acetic anhydride was added dropwise to 1% of the NBS. The reaction was incubated for 4 h at 80 °C. Subsequently, the reaction was cooled to room temperature, followed by ethanol precipitation. The precipitate was dried and dissolved in distilled water, and dialyzed against tap water for 24 h, followed by distilled water for 24 h using 3600 Da Mw cutoff dialysis membranes. The resulting mixture was concentrated and lyophilized to yield acetylated FPS (AF).

DFPS was acetylated according to the above conditions and was labeled as ADF.

The benzoylated FPS (PHF) and benzoylated DFPS (PHDF) were prepared according to a similar procedure, except that acetic anhydride was replaced with phthalic acid anhydride.

2.5. Phosphorylation of fucoidan [19]

$POCl_3$ (4 mL) was slowly added dropwise to 30 mL of pyridine and stirred for 15 min at 0–2 °C (ice-water bath). An FA solution (50 mL) containing FPS (2 g) was subsequently dripped in. The mixture was stirred for 3 h in an ice-water bath. To terminate the reaction, the mixture was poured into 150 mL of saturated aqueous $Ba(OH)_2$, and the resulting white precipitate was filtered off and washed with water. The filtrate