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Preparation of low molecular weight fucoidan by gamma-irradiation and its anticancer activity

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ABSTRACT

Fucoidan is a marine sulfated polysaccharide with a wide variety of biological activities. Recently, it has been reported that low molecular weight fucoidan has the enhanced antioxidant and anticoagulative activities. However, degradation techniques such as enzymolysis and acid hydrolysis for obtaining low molecular weight fucoidan, have the disadvantages such as narrow substrate specificity and unfavorable hydrolysis of side groups, respectively. In this study, low molecular weight fucoidan was prepared by gamma-irradiation. When fucoidan was gamma-irradiated, the molecular weight rapidly dropped to 38 kDa when the sample was irradiated at 10 kGy, then gradually dropped to 7 kDa without the significant elimination of the sulfate groups. Low molecular weight fucoidan had higher cytotoxicity than native fucoidan in cancer cells, such as AGS, MCF-7, and HepG-2. In addition, low molecular weight fucoidan showed higher inhibitory activity of cell transformation, which resulted in higher anticarcinogenicity. This result suggests that low molecular weight fucoidan with enhanced biological activities can be produced by a simple irradiation method without changing the functional groups.

1. Introduction

Fucoidan, a sulfated polysaccharide containing a substantial percentage of fucose and small amounts of galactose, xylose, mannose, and uronic acid, is a constituent of brown seaweed. For the past decades, fucoidans isolated from different species have been extensively studied because of their various biological activities, such as anticoagulant and antithrombotic, antiviral, and gastric protective properties (Choi et al., 2010; Li, Lu, Wei, & Zhao, 2008). A number of studies have shown that fucoidan suppressed the growth of tumor cells in *in vitro* and *in vivo* models. It has been suggested that the anticancer activity of fucoidan is attributable to the activation of the immune system or the inhibition of tumor growth by suppressing angiogenesis.

The biological activities of fucoidans are closely related to their molecular structures, which include fucose linkage, the sugar type, sulfate content, and molecular weight. Among these, molecular weight is one of the most important factors determining the biological activities of polysaccharides. High molecular weight polysaccharides may cause low solubility and processability, thereby hampering their penetration into the cell to perform a function. On the contrary, low molecular weight sulfated cellulose shows higher antioxidative and anticoagulation activities (Wang, Zhang, Zhang, Song, & Li, 2010a; Wang, Xiao, Li, & Wu, 2010b). In addition, low molecular weight fucoidan promotes revascularization of hindlimb ischemia in rats (Luyt et al., 2003), boosts osteoblast proliferation for bone regeneration (Igondjo Tchen Changotade et al., 2008), and enhances human endothelial cell formation (Chabut et al., 2003; Lake et al., 2006).

Low molecular weight fucoidan can be produced by acidolysis and enzymolysis, however, acidolysis is difficult to obtain the proper size of fucoidan and the sulfate groups in fucoidan can be removed because of the hydrolysis properties with acid. Pomin et al. (2005) reported that acid hydrolysis tends to selectively desulfate at the 2 position of the first fucose unit and then the glycosidic linkage between nonsulfated fucose residue and the subsequent 4-sulfated residue is preferentially cleaved by acid hydrolysis. Fucoidanase has been found in marine bacteria including *Pseudomonas*, *Bacillus*, and *Vibrio*, and marine fungi (Bakunina et al., 2000). However, the activity of fucoidanase is quite variable depending on the molecular structure of fucoidan, and no conventional fucoidanse degrades all the different structures of fucoidan. In addition, the production of low molecular weight fucoidan less than 10 kDa, needs a complex enzymatic process (Wu et al., 2010). There are several reports showing that the low molecular weight polysaccharides degraded by gamma-irradiation enhance biological activities. When β-glucan is degraded by irradiation, its antioxidant and immune-enhancing activities increase, depending on the irradiation dose (Byun et al., 2008; Sung et al., 2009). The antioxidative activity of laminarin, another polysaccharide in

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seaweed, also increases after gamma-irradiation (Choi, Kim, & Lee, 2011). The benefits of radiation degradation include a short process period, with no addition of acid and enzyme, unnecessary purification after degradation or possible degradation of powder form, and simultaneous sterilization.

Therefore, in this study, fucoidan was degraded by gamma-irradiation and its biological activity was investigated on inhibitory activity in different types of cancer cells and its anticarcinogenic activity.

2. Materials and methods

2.1. Materials

Fucoidan was purchased from Sigma Chemical Co. (St. Louis, MO), which was originated from *Fucus vesiculosus*. Samples were dissolved at 10 mg/mL (w/v) in double distilled water for the gamma-irradiation. Other constituents such as proteins and uronic acid in fucoidan samples were determined because these possibly involve in highly biological response. Uronic acid content was shown to be 11.6 (mass%) determined by carbazole reaction (Bitter & Muir, 1962), Also, protein concentration was about 1.2% quantified by Bradford method (1976). The contents of uronic acid and proteins in fucoidan samples used in this study was almost same as in other purified fucoidan samples (You, Yang, Lee, & Lee, 2010).

Eagle's minimal essential medium (MEM), Roswell Park Memorial Institute (RPMI-1640) medium, fetal bovine serum (FBS), penicillin, and streptomycin were purchased from Invitrogen (Carlsbad, CA, USA). 12-*O*-tetradecanoylphorbol-13-acetate (TPA) was purchased from Calbiochem-Novabiochem (San Diego, CA, USA).

2.2. Preparation of degraded fucoidan with gamma-irradiation

The dissolved fucoidan (10 mg/mL) was gamma-irradiated in a ^{60}cobalt gamma-irradiator (IR-221, Nordion International Ltd., Ontario, Canada) with a strength of 11.1 petabecquerel (pBq) at $22 \pm 2\,^{\circ}C$ at a dose rate of 10 kGy/h. The applied dose levels were 10 kGy, 30 kGy, 50 kGy, and 100 kGy, respectively. Dosimetry was performed with 5 mm-diameter alanine dosimeters (Bruker Instruments, Rheinstetten, Germany). After the gamma-irradiation process, the samples were stored at 4 °C for further experiments.

2.3. Gel permeation chromatography (GPC) analysis

GPC was performed using a following system; separation module (Waters 2690, Waters Co., Milford, MA), refractive index detector (RI, Waters 2410, Waters Co.), Empower software (System Software, Empower option GPC, Waters Co.), and PL aquagel-OH -60, -40, and -30 columns (300 mm × 7.5 mm, 8 μm, Polymer laboratories Ltd., UK). The mobile phase was 0.1 M sodium nitrate at the flow rate of 1 mL/min, and the analyses were performed at 40 °C. The injection volume was 200 μL (10 mg/mL fucoidan), and calibration was carried out using pullulan standard (Showa Denko K.K., Tokyo, Japan) (Choi et al., 2011).

2.4. Determination of sulfate contents

The content of sulfate in fucoidan was determined by the method using 1,9-dimethyl-methylene blue (DMMB) (Farndale, Buttle, & Barrett, 1986). The sample solution (10 mg/mL fucoidan, 0.1 mL) was added to 0.2 mL of DMMB reagent (16 mg of DMMB, 3.04 g of glycine, and 2.37 g of sodium chloride per 1 L). The reaction mixture was vortexed for thorough mixing. The absorbance was measured using a spectrophotometer (UV-1601PC, Shimadzu Co., Tokyo, Japan) at 525 nm. Quantification was done based on a standard curve generated with chondroitin-6-sulfate (Sigma Chemical Co.).

2.5. Cytotoxicity in cancer cells

Human breast cancer cells (MCF-7), human stomach cancer cells (AGS), and human liver cancer cells (HepG-2) were purchased from the Korean Cell Line Bank (Seoul, South Korea) and adapted in RPMI-1640 medium containing 10% FBS, 100 unit/mL penicillin and 100 unit/mL streptomycin and then cultured at 37 °C in 5% CO_2. Types of cancer cells were seeded into 96-well-plate with 3×10^4/well, respectively, and fucoidan samples were added in the plate at different doses. After 24 h incubation, cancer cell cytotoxicity was detected by the MTT [3-(4,5-dimethylthiazolyl)-2,5-diphenyl-tetrazolium bromide] (Sigma Chemical Co.) assay method. Briefly, 30 μL of 5 mg/mL MTT reagent dissolved in PBS was added to each well and the plate was incubated at 37 °C. After 2 h, each plate was then centrifuged and the medium was removed. One hundred microliters of dimehylsulfoxide (Sigma Chemical Co.) was then added. After incubation at 37 °C for 5 min, the absorbance from fucoidan treated and non-treated cells was measured at 517 nm by micro-plate reader (Zenyth 3100, Anthos Labtec Instrumentrs GmbH, Salzburg, Austria). The percentage of cell cytotoxicity was calculated the following equation,

$$\text{Cytotoxicity} = 100 \times [1 - (A_s/A_c)],$$

where A_s is the absorbance with fucoidan sample and A_c is the absorbance of control for which H_2O was used instead of sample solution.

2.6. Anchorage-independent transformation assay

JB6 Cl41 mouse epidermal cells were cultured in MEM supplemented with 5% FBS. TPA-induced cell transformation was investigated in JB6 Cl41 cells. In brief, cells (8×10^3/mL) were exposed to TPA (10 ng/mL) with or without fucoidan (1 μg/mL or 10 μg/mL) in 1 mL of 0.3% Basal Medium Eagle (BME) agar containing 10% FBS, 2 mM L-glutamine, and 25 μg/mL gentamicin. The cultures were maintained at 37 °C in a 5% CO_2 incubator for 10 days, and the cell colonies were scored using a microscope and the Image-Pro PLUS computer software program (Media Cybernetics, Silver Spring, MD) as described by Lee et al. (2008a, 2008b).

2.7. Statistical analysis

All of the experiments were carried out 3 times. For molecular weight and cytotoxicity experiments, one-way analysis of variance was carried out using the SPSS software system, and the Duncan's multiple-range test was used to compare the differences among the mean values. For the comparison of cytotoxicity and carcinogenetic inhibitory effect of fucoidan, student's *t*-test was performed with $p < 0.05$ for statistical significance.

3. Results and discussion

3.1. Degradation of fucoidan with gamma-irradiation

The change in the molecular weight of fucoidan by the gamma-irradiation is shown in Fig. 1. The weight average molecular weight decreased as the irradiation dose increased. When the irradiation dose was 10 kGy, the molecular weight of fucoidan decreased to 38 kDa from 217 kDa of the non-irradiated fucoidan. The molecular weights of fucoidan after irradiation at the doses of 30 kGy, 50 kGy, and 100 kGy were 16 kDa, 10 kDa, and 7 kDa, respectively. The molecular weight loss of fucoidan by irradiation was similar to