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Effect of gamma irradiation on structure, physicochemical and immunomodulatory properties of *Astragalus* polysaccharides

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ABSTRACT

Astragalus polysaccharides (APS) were treated with different gamma irradiation doses (10, 25, 50, 100 and 150 kGy) to investigate the effects of gamma radiation processing on structure, physicochemical and immunomodulatory properties. The results revealed both the number-average and weight-average molecular weight of APS significantly decreased with increasing irradiation dose, whereas the solubility was increased after irradiation. A decrease in the apparent viscosity, as well as an increase in amount of small fragments of APS granules was also observed with increasing irradiation dose. FT-IR spectra indicated that gamma irradiation introduced no significant changes into the functional group status of APS. High irradiation dose (>50 kGy) caused a significant increase of yellowness and a slightly decrease of thermal stability of APS. Further, the immunomodulatory activity of irradiated APS was evaluated on Caco2 cells. APS irradiated at dose of 25 kGy exhibited the highest ability to induce nitric oxide production and up-regulate the mRNA expression of inflammatory cytokines, occludin, zonula occludens protein-1 (ZO-1) and toll-like receptor 4 (TLR4), as well as the protein expression of ZO-1 and TLR4. These findings indicate that gamma irradiation modification with a proper dose enhance immunomodulatory activity of APS by improving physicochemical properties without changing the functional groups.

1. Introduction

Astragalus polysaccharides (APS) is the major active component of the radix of *Astragalus membranaceus*, which, as a commonly used Chinese medicine for nearly 2000 years, has already been officially documented in the Chinese pharmacopoeia (2005 version). Over the past several decades, APS has been extensively studied because of its various biological activities, such as anti-inflammatory [1], anti-bacterial [2], anti-oxidant [3,4], anti-tumor [5] and especially immunomodulatory activity [6,7]. APS enhances concanavalin A-induced proliferation of peripheral blood lymphocytes, increases $CD4^+$ to $CD8^+$ ratio, and potentiates immune system development [8]. APS also promotes the production of antibodies, activates the complement system, changes the expression of cell messenger molecules, and synergize antibodies to kill pathogenic microorganisms, and thereby enhances the body immunity [9]. However, the application of APS is often confined since its large adding amount and high cost [10,11]. Therefore, many researchers have made an effort to explore effective ways to improve the physiological activities of APS through structural modification.

Recently, gamma radiation has been considered as a useful physical method for the modification of polysaccharides, such as β-glucan, laminarin and fucoidan, through cross-linking, grafting, and degradation [12–14]. There are several reports showing that irradiation leads to the cleavage of glycosidic bonds by electromagnetic waves of gamma rays, which subsequently results in low molecular weight polysaccharide products [12,14,15]. Basic advantages of radiation degradation of polymers include ability to promote changes reproducibly and quantitatively without the introduction of chemical reagents, and without the need for special equipments or setups to control temperature, environment and additives [16]. Therefore, physical modification of polysaccharides involving gamma irradiation is a fast, low cost and environmental friendly way in comparison to complex chemical modification. Besides, appropriate sterilization may be also accomplished in parallel to the reduction in molecular weight of polysaccharides by irradiation degradation [17].

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The biological activities of polysaccharides are closely related to their molecular structures, which include glycosidic bond types, spatial conformation of the bonds, the degree of polymerization and molecular weight. Among these, molecular weight is one of the most important factors affecting the biological activities of polysaccharides [14]. Generally, high molecular weight polysaccharides may cause low solubility and high viscosity, thereby hampering their penetration into action sites to perform a function. In previous studies, gamma irradiation clearly elevated the immune-enhancing of β-glucan, and the modification effect was a dose-dependent manner [18,19]. The same go for the anticancer activity of fucoidan [14]. Thus, we speculate that irradiation modification is a potentially effective technique to obtain APS with high immunomodulatory activity. Therefore, the present study was undertaken to investigate the effects of gamma irradiation on structure and physicochemical modification of APS, and its immunomodulatory activity was also evaluated *in vitro*.

2. Materials and methods

2.1. Materials and reagents

APS was obtained from Tianjin Sainuo Pharmaceutical Co. Ltd. (Tianjin, China). The content of polysaccharides was 89.3% as determined by the phenol-sulfuric acid assay [20]. Dextran, penicillin-streptomycin solution, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), trypsin were purchased from Gibco GRL (Grand Island, NY, USA). Cell culture dishes were purchased from Corning Inc. (Corning, NY, USA). RNAiso Plus reagent, PrimeScript@RT Master kit and SYBR Premix Ex Taq II kit were purchased from TaKaRa Biotechnology Co., Ltd. (Dalian, China). Rabbit anti-occludin (ab167161) and mouse anti-GAPDH (ab8245) monoclonal antibodies were purchased from Abcam (Cambridge MA, UK). Rabbit anti-ZO-1 (8193T) and mouse anti-TLR4 (sc293072) monoclonal antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA) and Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA), respectively. The other chemicals used in the experiment were of analytical grade.

2.2. Gamma radiation treatment

The APS samples were packed in polyethylene bags and were irradiated using a BFT-IV cobalt-60 source irradiator at an ambient temperature of 25 ± 0.5 °C in the XiYue Irradiation Technology Co., Nanjing, China. The irradiation doses applied in this study were 0, 10, 25, 50, 100 and 150 kGy. The source strength was 2×10^6 Ci with a dose rate of 4 Gy/s. Both the native and irradiated APS samples were stored at −20 °C for further use. All structural and physicochemical analyses were accomplished within one month after irradiation.

2.3. Color measurement

The color of native and irradiated APS samples were measured using Chroma Meter CR-400 optical sensor (Konica Minolta Sensing, Inc., Osaka, Japan) and expressed on the *L*, *a* and *b* tristimulus scale [21].

2.4. Gel permeation chromatography (GPC) analysis

The molecular weight distributions of the native and irradiated APS samples were determined by gel permeation chromatography using Water 515 gel permeation chromatography (Waters System, Milford, MA, USA) equipped with a BIOSEP G4000SWXL (7.8 × 300 mm, Tosoh Co., Tokyo, Japan) column, and a Water 2410 refractive index detector. The columns were eluted with 0.1 M nitric acid and calibrated with standard dextran. The number-average molecular weight (Mn), weight-average molecular weight (Mw) and polydispersity index (Mw/Mn) were calculated using molecular weight calculation software connected to the gel permeation chromatography system.

2.5. Solubility determination

To investigate the solubility of the native and irradiated APS, 40 mg of sample was dissolved into 0.4 mL of deionized water, and simply vortexed for 30 min. The solution was centrifuged at 3500 rpm for 30 min and then supernatant was removed. The remaining sediment was collected and dried at 100 °C for 0.5 h using a dryer. The solubility was calculated based on the following equation as follows:

$$\%\text{Solubility} = [1-(\text{weight of dried sediment}/\text{weight of initial polysaccharides})] \times 100\%.$$

2.6. Viscosity measurement

The native and irradiated APS samples were dissolved in distilled water to a certain concentration (10%, w/v) and then used a Physical MCR 301 rheometer (Anton Paar GmbH, Graz, Austria) to determine the viscosity at 25 °C. The diameter and gap of the parallel-plate system were 20 mm and 0.5 mm, respectively. Viscosity measurements were the average of at least 3 determinations.

2.7. Thermal properties analysis

The thermal properties of the native and irradiated APS samples were carried out using a DSC822 differential scanning calorimeter (DSC) (Mettler Toledo, Columbus, USA). A 3.7 mg sample was sealed in a standard aluminum dish, and, meanwhile, a sealed empty aluminum dish was used as the reference sample. Sample was scanned from 20 °C up to 350 °C at a ramping rate of 10 °C/min. Nitrogen was used as a purging gas (50 mL/min). Characteristic temperatures of the transitions were recorded, including glass temperature (T_g), crystallization temperature (T_c), melting temperature (T_m), crystallizing enthalpy (ΔH_c) and melting enthalpy (ΔH_m).

2.8. Fourier transform infrared (FT-IR) spectrometry analysis

FT-IR spectra of the native and irradiated APS samples were recorded on a Nicolet 380 FT-IR spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Samples were prepared as thin films, and mixed with KBr. Spectra were obtained in the wavelength range of 4000–500 cm^{-1} with a resolution of 4 cm^{-1}, and the measurements were averaged on 16 scans.

2.9. Scanning electron microscopy

The microstructural changes between the native and irradiated APS samples were observed with a Hitachi SU8010 scanning electron microscope (Hitachi High Tech Co., Tokyo, Japan). The dried samples were mounted on cylindrical microscope stub covered with carbon strip and coated with a thin layer of gold for 15 s before observing.

2.10. In vitro immunomodulatory activity analysis

2.10.1. Cell lines and culture

Human colon cancer cell line Caco2 cells were obtained from the Cell Bank of Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China) and cultured in DMEM supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin at 37 °C in a humidified incubator (Thermo Fisher Scientific) with 5% CO_2. Cell culture media were changed every 2 days. The cells were passaged every 3–5 days using 0.20% EDTA trypsin solution.