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Physicochemical properties and cellular protection against oxidation of degraded *Konjac* glucomannan prepared by γ-irradiation

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ABSTRACT

Konjac glucomannan (KGM) is an important functional polysaccharide in food research. However, unstable dispersibility of KGM inhibits its in-depth study and wide application. In this study, a degraded KGM (100 kGy-KGM), which showed excellent dispersibility and specific physicochemical properties, were obtained by γ-irradiation in a dosage of 100 kGy. We investigated the protective effect of 100 kGy-KGM against H_2O_2 induced oxidative damage in LO2 cells. Our results demonstrated that pretreatment of LO2 cells with 100 kGy-KGM not only significantly increased cellular survivals and activities of GSH-Px and CAT, but also reduced levels of LDH, MDA and intracellular accumulation of ROS. The marked protective effect against oxidative damage and excellent dispersibility in 100 kGy-KGM allowed its possible use as an antioxidant. Our study provided fundamental knowledge to understand the structure-functions relationships of degraded-KGM, which could result in a theoretical guidance for the future application of KGM.

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1. Introduction

Reactive oxygen species (ROS) is a critical pathogenic factor involved in cell injury and various disorders, such as cancer, neurodegenerative diseases, autoimmune diseases, among others (Li et al., 2010). Some common ROS, including hydrogen peroxide, super oxide anion and hydroxyl radical, are usually observed in biological system and need to be eliminated (Yu-Jing et al., 2010). Therefore, much attention has been focused on antioxidants, which could attenuate oxidative damage of a tissue by enhancing natural defenses of cell through enhancing enzymatic conversion of ROS or scavenging the free radical species (Shokoohinia, Rashidi, Hosseinzadeh, & Jelodarian, 2015). Besides the common antioxidants, such as vitamin E, flavonoids and phenolic acids, some natural polysaccharides have been ascertained to exhibit protection against oxidative damage induced by H_2O_2 *in vitro* cells or *in vivo* tissues (Liu, E, Zu, Tao, & Liu, 2013). Polysaccharides from plants, animals and microorganisms have been regarded as a promising group of antioxidative compounds.

Detailed molecular properties of relevant polysaccharides have not been fully studied (Kozarski et al., 2014). Up to now, most polysaccharides, which were tested for antioxidant activities, were

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crude or partially purified polysaccharides fractions with complex or unknown chemical composition (Siu, Xu, Chen, & Wu, 2016). The exact relationship between chemical structure and anti-oxidation activity of pure polysaccharide remains uncertain. As verified in previous researches, the structure characteristics, such as monosaccharide composition, degree of branching, molecular weight and conformation, plays a vital role on bioactivities of polysaccharides (Zhang, Cui, Cheung, & Wang, 2006). It is, therefore, essential to investigate the anti-oxidation of polysaccharide fractions with specific molecular properties, so as to establish the relationship between molecular properties and anti-oxidation.

Konjac glucomannan (KGM), a plant polysaccharide derived from the tuber of *Amorphophallus konjac* C. Koch, has been fully elucidated about its chemical structure and composition. It is composed of glucose and mannose, at 1:1.5–1:1.6 M ratios, with the linkage of β-1, 4 glycosidic bond and 5–10% acetyl substitution (Jian, Siu, & Wu, 2015). KGM has been widely studied in the food and pharmaceutical field for its various biological activities, including anti-tumor, immunomodulation and wound healing (Chua, Baldwin, Hocking, & Chan, 2010). Several health benefits have also been found in KGM, such as anti-obesity, anti-hyperglycemic, anti-inflammatory and prebiotic activity (Alonso-Sande, Teijeiro-Osorio, Remuñán-López, & Alonso, 2009).

Though many biological activities and health benefits are found in KGM, native KGM is easy to swell, precipitate and form gels in dispersion due to their high molecular weight (about 1000 kDa) and strong water-absorbing capacity (Chua et al., 2010). It is difficult to maintain the stable dispersion of native KGM even in extremely low concentration at room temperature or upon thermal treatment, which greatly inhibits the in-depth study on biological activity and development as health products. Thus, it is necessary to improve the dispersibility of native KGM by modification. Previous studies showed that degradation by γ-irradiation with suitable dosages may be an effective way to obtain degraded-KGM with good dispersibility and without touching its basic chemical composition (Xu, Sun, Yang, Ding, & Pang, 2007).

Besides the significant improvement on dispersibility of native KGM, degradation may also induce the new health benefits. Recent studies demonstrated that, comparing to native KGM, degraded or hydrolyte of KGM showed some more new functions or higher activity (Tester & Al-Ghazzewi, 2013). The hydrolysates of KGM displayed significant improvement on bowel movement and effective therapy effect on inflammatory bowel disease (Suwannaporn et al., 2013). Higher prebiotic and anti-obesity effects were also found in degraded and pulverized KGM (Suzuki et al., 2010). Furthermore, partially-hydrolyzed KGM exerted greater protective effects than native KGM on fecal water-induced DNA damage (Yeh, Lin, & Chen, 2010). These results signalled that degraded KGM may also protect against oxidative damage in cells induced by hydrogen peroxide, despite no reports in native KGM.

This study aims to obtain properly-degraded KGM with excellent dispersibility, with suitable dosages of γ-irradiation. Protective effect of degraded KGM was examined against oxidative damage induced by hydrogen peroxide.

2. Materials and methods

2.1. Raw materials and chemicals

KGM was purchased from Shaotong Shanai *Konjac* Development Co. (Yunnan, China), which was extracted from the corm of *Amorphophallus konjac* C. *Koch*. After being further purified by alcohol sedimentation (Jian et al., 2016), the purity of KGM was up to 95%. This purity was reasonable for biological testing, as similar purities (94–97%) were reported by literature (Yeh et al., 2010), in which biological activities of KGM were evaluated (Suzuki et al., 2010).

LO2 cell line (Human hepatic cell line) was provided by Prof. Hu-lin Jiang from China Pharmaceutical University. Major chemicals, such as Dulbecco's modified Eagle medium, fetal bovine serum, penicillin and streptomycin, were purchased from certified suppliers with purity up to 99.99%.

2.2. Preparation of degraded-KGM

Based on our previous study (Jian et al., 2016), the degraded KGM was obtained by γ-irradiation, using the purified KGM as raw materials. After being sealed in polyethylene bags and canned, in a tin can, the purified KGM was irradiated with a ^{60}Co source. Afterwards, they were stored in desiccator at room temperature. The applied dosage for γ-irradiation was 10 kGy, 20 kGy or 100 kGy, respectively. Based on the irradiation dosage, the sample was labelled as native, 10 kGy-, 20 kGy- or 100 kGy-KGM, respectively.

2.3. Chemical composition determination

Referring to literature (Jian et al., 2015), the following classical methods were used to analyze the chemical composition of KGM samples, and the details were reported in our previous work (Jian et al., 2016). Infrared (IR) measurement was performed using the method of KBr slices, on a Fourier transforms IR spectrometer in the range of 400–4000 cm^{-1} at a resolution of 4 cm^{-1}. After acid hydrolysis and derivatization, the monosaccharide composition was determined by HPLC. Titration using hydrochloride solution was applied to measure the acetyl content. In regard to the determination of sugar and protein content, phenol sulphuric acid method and kjeldah method were used, respectively.

2.4. Molecular characteristics characterization

As reported previously (Jian et al., 2015), the determination of molecular characteristics (molecular weight, root-mean-square radius, and molecular conformation) and second virial coefficient (A_2) of KGM was performed by gel permeation chromatography (GPC) conjugated with on-line multi-angle laser light scattering (MALLS) on e2695 HPLC system (Waters, USA) with DAWN HELEOS II detector (Wyatt, USA). The HPLC system included columns of TSK-GEL G4000PWxl and G5000PWxl (Tosoh Bioscience, Tokyo, Japan), and a refractive index detector (Waters 2414, USA). The solution of sodium nitrate (50 mM) with sodium azide (0.02% w/v) was used as mobile phase at a speed of 0.35 ml/min. The temperature of the columns and refractive index detector was controlled at 40 °C. Before determination, the GPC-MALLS system was normalized with dextran standards (Sigma Aldrich, Mw 15 kDa). After being fully dispersed and centrifuged (12,000 rpm, 4 °C, 30 min), 100 μl of sample dispersion (0.25 mg/ml) was loaded into the system for measurement. Astra 6.0 software package was applied to collect and analyze the measurement data, with the value of dn/dc set at 0.147 ml/g.

2.5. Cell culture

LO2 (Human hepatic cell line) cells were cultured in newly-prepared Dulbecco's modified Eagle medium (DMEM) (Gibco, NY, USA) with 10% fetal bovine serum (FBS) (Gibco, NY, USA), penicillin (100 U/ml), and streptomycin (100 μg/ml) (Gibco) under a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. To maintain the reliability of the LO2 cell, during the whole experiment, the cell was stored in ultra-low temperature refrigerator (BCD-348WA/H,

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