DOCTORAL THESIS

Effect of molecular weight and structure on anti-inflammatory properties of polysaccharide from submerged mycelial fermentation of schizophyllum commune

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Abstract

Medicinal mushrooms are therapeutic agents in traditional folk medicines. Previous studies have shown that a number of biologically active compounds in medicinal mushrooms contributed therapeutic functions against many diseases. These compounds include mainly large molecular weight (MW) compounds such as polysaccharides, dietary fibre and lipids. Mushroom polysaccharides have attracted great attention in food and pharmacology fields because of their biological activities. Polysaccharides vary in molecular weight, degree of branching and conformational structure. It has been reported that fine structure, molecular weight, and conformation of polysaccharide influence biological activities. The incidence and prevalence of inflammatory bowel disease (IBD) have been increasing worldwide, which is characterized by chronic inflammation of the gastrointestinal tract but without satisfactory treatment. Although there are many studies for the immuno-pharmacological activity of mushroom polysaccharides, their intestinal anti-inflammatory property has not been investigated sufficiently. Therefore, it is very important to elucidate whether there is the relationship among the MW, structure and anti-inflammatory activity of polysaccharide in IBD.

Firstly, an exopolysaccharide from a mycelial culture of *S. commune* was obtained by isolation and purification using DEAE–52 cellulose and Sephadex G–150 column chromatography. The structure, conformation and chemical properties were investigated, including elemental compositions, MW, monosaccharide compositions, fourier transform infrared spectrum, thermogram analysis, nuclear magnetic resonance (NMR) spectrum, circular dichroism (CD) study, methylation analysis, and scanning electron microscope (SEM). The findings indicate that the exopolysaccharide is a homogeneous protein-bound heteropolysaccharide carrying molecular weight of 2900 kDa with a *β*-type glycosidic linkage. It belongs to a kind of *β*-(1→3)-*D*-glucans consisting of a backbone of *β*-(1→3)-linked glucose residues branched with (1→4) and (1→6)-*β*-*D*-glucopyranosyl residues on main-chain residues. The elemental analysis of this exopolysaccharide discover the element compositions as: C, 25.84%; H, 5.45%; and N, 0.65%. The total carbohydrate, protein and uronic acid contents of exopolysaccharide is 89.0%, 2.20% and 7.52%, respectively. In addition, lipopolysaccharide (LPS) was not detected in the exopolysaccharide. Glucose is the main monosaccharide structural unit in this exopolysaccharide, the content is 57.5%. The degradation temperature of exopolysaccharide is 278.9 ℃ from the thermogram analysis curve. This exopolysaccharide looks like thin film with smooth and glittering surface in SEM photography. It is clear from these images that the exopolysaccharide is linear in structure and branched and coiled in aqueous solution. With these extraction, the preliminary anti-inflammatory activity of *S. commune* exopolysaccharide was conducted by inhibiting the production of nitric oxide (NO), activity of inducible nitric oxide synthase (iNOS) and activity of 5-lipoxygenase (5-LOX) from RAW 264.7 macrophages. The

results showed that exopolysaccharide significantly inhibit LPS-induced iNOS expression levels in a dose-dependent manner($p < 0.05$). It inhibits the production of 5-LOX in cells, but not in dose-dependence. Further, in dextran sulfate sodium (DSS)-induced colitis model, the results showed that exopolysaccharide attenuated body weight loss, diarrhea, fecal blood, and the shortening of colon and improved histological changes. Furthermore, exopolysaccharide treatment would reduce NO production and some cytokines' secretion such as IL-4 and IL-17A. These results indicate that exopolysaccharide might be exploited as an effective anti-inflammatory agent for application in IBD.

Secondly, ultrasound technology was applied to modify the physicochemical properties (MW and viscosity) of this fungal exopolysaccharide, and fractions of different MWs were obtained through ultrasonic degradation method. Effect of the MW degradation, viscosity and anti-inflammatory property of exopolysaccharide under ultrasonic treatment were optimized with response surface methodology. The best ultrasonic treatment parameters were obtained with a three-variable-three-level Box-Behnken design. The optimized conditions for efficient anti-inflammatory activity include: Initial concentration – 0.4%; ultrasonic power – 600 W; and duration of ultrasonic treatment – 9 min. Under these conditions, the NO inhibition rate is $95 \pm 0.03\%$ which agreed closely with the predicted value (96%). Average MW of exopolysaccharide decreased after ultrasonic treatments, but no significant change in the preliminary structure by infrared spectroscopy analysis. The viscosity of degraded exopolysaccharide dropped compared with native exopolysaccharide. The results suggest that ultrasound technology is an effective approach to reduce the MW of exopolysaccharide. Our results also showed that exopolysaccharide from *S. commune* was degraded into three fractions (low, medium, and high MW) by ultrasonic treatment. The changes of MW, atomic force microscope morphology, X-ray diffraction, particle size distribution and viscosity analysis indicate the triple helical structure of exopolysaccharide was dissociated into single helical structure and random coiled structure by breaking of inter- and intramolecular hydrogen bonds. The medium and high MW exopolysaccharide had the mixture of triple helix and single helix conformation. Moreover, the low MW exopolysaccharide exhibit random coiled conformation. As for their anti-inflammatory effect in DSS-induced colitis mice model, the results showed that medium and high MW exopolysaccharide significantly recovered DSS-induced colitis in body weight loss, shortening of colon lengths, colon weight loss, diarrhea and rectal bleeding, histological score, myeloperoxidase (MPO) activity, NO and cytokines (IFN-γ, IL-10 and IL-17) production in inflamed tissues. Moreover, exopolysaccharide with medium and high MW reduced DSS-induced infiltration of macrophages. These results showed that medium and high MW exopolysaccharide had intestinal anti-inflammatory activity. The degraded exopolysaccharide with medium and high MW had a triple and single-helical structure. These results suggested that the intestinal

anti-inflammatory activity of exopolysaccharide from *S. commune* is related to both helical structure and MW.

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