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Structural Characterization of an Immunological Polysaccharide from Floral Mushroom Grown in Huangshan Mountain

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ABSTRACT

Floral mushroom was a wood-rotting fungus. A homogeneous polysaccharide, named FMP2-1, was extracted from floral mushroom grown in the Huangshan Mountain and further purified by DEAE-cellulose and Sephacryl S-300 column chromatography. Monosaccharide analysis showed that FMP2-1 was glucan. The structural features of FMP2-1 were carried out using methylation analysis, infrared spectroscopy (FT-IR) and nuclear magnetic resonance spectroscopy (NMR). The results revealed that FMP2-1 had a backbone chain consisting of a (1→4)-α-D-Glcp and (1→6)-β-D-Glcp with the branched chain T-β-D-Glcp attached to the O-6 position of (1→4)-α-D-Glcp. Immunostimulatory activity was measured via murine macrophage cell line RAW264.7 cells in vitro. The FMP2-1 exhibited significant in macrophage proliferation, NO production and phagocytic activity with optimum dose of 200 μg/mL, 100 μg/mL and 200 μg/mL, respectively. Therefore, the deletion of branching and reduction of average molecular weight caused by partial acid hydrolysis was demonstrated to weaken the immunomodulatory activity of FMP2-1. Results suggested FMP2-1 could be a potential immunomodulatory agent for food industry.

Keywords: Polysaccharide; Structural properties; Immunological; Floral mushroom

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INTRODUCTION

Fungal polysaccharides, extracted from fungi, were important components of bioactive substances [1]. Structural features and immunological activities of fungal polysaccharides were extensively studied. Glucans were the most widely detected fungal polysaccharide in previous reports [2,3]. Based on their anomeric structure of D-Glc*p* units, it was possible to represent three main types: α-D-glucans, β-D-glucans and mixed α,β-D-glucans [4,5]. In addition to glucans, mannans, xylans and galactans were quite common in fungal polysaccharides [6]. Moreover, the immunological activities of glucans from fungi were highly influenced by structural features. The structure of fungal polysaccharides was related to the raw materials and extraction methods [7,8].

As an important bioactive ingredients in nature, polysaccharides exhibited biological activities such as immunomodulating [9], anticomplementary [10], anticoagulant [11] and antitumor [12], which probably could be related to monosaccharide composition, glycosidic linkage, molecular weight, conformation, functional group and branching degree [13-15]. Part of fungal polysaccharides can interact direct or indirectly with macrophage, leading to the increased immunity of host [16,17]. However, systematic reports of structure–function relationships of fungal polysaccharides were still rare. Fungal polysaccharides were utilized widely due to its immunological activities. For instance, polysaccharides from *Lentinus edodes*, *Ganoderma lucidum*, *Coriolous versicolor* and *Grifola frondosa* as

active principle in food were commercialized. Hence, it is necessary to carry out more extensive studies on the structure of polysaccharides from different fungi species.

Huangshan floral Mushroom was an edible mushroom that grows in Huangshan Mountain (Anhui, China). It was a wood-rotting fungus that belongs to the family omphalotaceae of basidiomycetes and formed by changing the temperature, humidity, light and ventilation in the production process of *Lentinus edodes*. A systematic study on floral mushroom was conducted in our laboratory. In published articles, an alkali-extracted polysaccharide fraction was reported [18,19]. We described a novel water extraction polysaccharide with immune activity this time. In this paper, a novel water-soluble polysaccharide fraction (FMP2-1) have been isolated from the aqueous extract of floral mushroom and purified through a DEAE-cellulose column chromatography and a Sephacryl S-300 column chromatography. The structural characterization of FMP2-1 was elucidated by infrared spectroscopy (FT-IR), gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR). In addition, the immunomodulatory activity of FMP2-1 was evaluated by assaying proliferation, phagocytic activity and nitric oxide (NO) production of RAW264.7 macrophages. The results would provide meaningful data to develop novel supplementary for food industry.

EXPERIMENTAL SECTION

Materials and Reagents

Floral mushroom were purchased from Huangshan city, Anhui province, China. DEAE-Cellulose, Sephacryl S-300, standard monosaccharides (D-glucose, D-mannose, D-xylose, L-galactose, L-rhamnose and L-arabinose), T-series dextrans (molecular weight 1.0 KDa, 2.0 KDa, 15.0 KDa, 40.0 KDa, 50.0 KDa and 200.0 KDa), dimethyl sulfoxide (DMSO), trifluoroacetic acid (TFA) were purchased from Sigma-Aldrich Co., Ltd. (St. Louis, MO, USA). Newborn calf serum was purchased from Sijiqing Co., Ltd. (Hangzhou, China). Penicillin-streptomycin solution was purchased from Hyclone Company. Dulbecco's modified Eagle's medium-high-glucose (DMEM /High-Glucose) were purchased from Thermo Fisher Scientific. All other reagents were of analytical grade.

Isolation and Purification of Polysaccharides

The dried floral mushroom was crushed into powder and then extracted using petroleum ether and acetone under reflux to remove lipids and liposoluble pigments. After organic solvent removed by air drying at 50℃, the residues were extracted twice using distilled water (1:35, w/v) for 2 h at 100℃.

The supernatants were collected and concentrated by rotary evaporator. The concentrated solution was precipitated with 80% ethanol to remove ethanol-soluble components. After discarding supernatant by centrifugation, the precipitate was re-dissolved in distilled water and removed proteins by Sevage method [20]. After the protein removal procedure, the polysaccharides solution was decolorized, dialyzed, concentrated and freeze-dried to obtain the water extractable polysaccharides from floral mushroom (FMP). The yield was 6.4%.

FMP was fractionated on a DEAE-52 column (2.6 cm × 40 cm) and eluted with a step-wise gradient of 0 M, 0.1 M, 0.2 M and 1.0 M NaCl at a flow rate of 2.5 mL/min. The eluates were collected at 4 min per tube by auto-fraction collector and named as FMP1, FMP2, FMP3 and FMP4, respectively. The major fraction (FMP2) was further purified by Sephacryl S-300 column chromatography (1.5 cm × 100 cm) eluted with ultrapure water at a flow rate of 0.2 mL/min (2 min/tube), yielding one single fraction named FMP2-1.

Chemical Properties Analysis

The total sugar content was quantified by the phenol-sulfuric acid method using glucose as a standard [21]. The content of protein was assessed by the Coomassie Brilliant Blue G-250 method [22]. The uronic acid content of polysaccharide was determined by *m*-hydroxydiphenyl method [23].

Determination of the Homogeneity and Molecular Weight

The homogeneity and molecular weight (Mw) of FMP2-1 was determined by using a high performance gel-permeation chromatography (HPGPC) with a Waters E2695 high performance liquid chromatography (HPLC) system and equipped with a Waters 2424 evaporative light scattering detector (Waters Corporation, USA). A Ultrahydrogel^{TM} 2000 column (7.8 mm × 300 mm) and a Ultrahydrogel^{TM} 500 column (7.8 mm × 300 mm) were connected in series. Sample at a concentration of 1 mg/mL was filtered through a 0.22 μm filtration membrane and injected with ultrapure water at 0.5 mL/min as mobile phase. A set of dextran standards (T-10, T-20, T-150, T-400, T-500, T-2000) have been used to determining their retention time in order to construct the standard curve.

Characterization of Polysaccharide Fractions

Fourier-transform infrared (FT-IR) spectroscopic analysis: FT-IR spectrometer (Nicolet 5700, Thermo Nicolet, USA) was used for recording characteristic groups. The sample (1.5 mg) was ground with KBr powder (200 mg) and then pressed into pellets at room temperature for FT-IR measurement in the wavelength range of 4000-400 cm^{-1} [24].

Gas chromatogram of monosaccharides composition: FMP2-1 (5 mg) was hydrolyzed with 2 M trifluoroacetic acid in a sealed ampere tube at 120℃ for 4 h. After completely removing the excess trifluoroacetic acid by vacuum rotary evaporator, sodium borohydride were added to the hydrolysates to reducing in room temperature for 3 h. The remaining sodium borohydride was treated with 25% acetic acid and water phase was eliminated in a rotary evaporator. 3.0 mL of acetic anhydride and pyridine were added to residue. After the reaction was performed in 110℃ for 1 h, the resulting alditol acetate derivatives were extracted and analyzed by GC.

Methylation analysis: According to the Hakomori method, methylation of FMP2-1 sample was performed [25]. Completely methylated polysaccharide was examined by FT-IR and the disappearance of the stretching band of O—H (3200-3700 cm^{-1}) was confirming complete methylation. The methylated FMP2-1 was depolymerized with 88% formic acid for 6 h at 100℃in an oil bath. After removing the excess formic acid by rotary evaporator, the following treatment procedures were same to the procedures mentioned above.

NMR analysis: The dried FMP2-1 (80 mg) was dissolved in 1 mL of D_2O and centrifuged before transferred to a 5 mm NMR tube. The ^{1}H NMR, ^{13}C NMR, HSQC and HMBC were recorded on a VNMRS600 NMR spectrometer (Agilent).

Partial acid hydrolysis: FMP2-1 was hydrolyzed with 8 mL of 0.5 M TFA at 100°C for 1 h. The TFA was evaporated with MeOH in a rotary evaporator completely. The residue was dialyzed against distilled water for 2 days (Mw cut off: 3500 Da). The retentate was collected, concentrated and freeze-dried. It was termed as FMP2-1S.

Immunostimulatory activity: Murine macrophage cell line RAW264.7 cells were provided by Professor Jian Liu (Hefei University of Technology, Hefei, China). RAW264.7 cells were maintained in DMEM/High-Glucose medium containing 10% NBCS (v/v), 100 IU/mL streptomycin and 100 IU/mL penicillin in a humidified atmosphere containing 5% CO_2 at 37°C.

Macrophage proliferation: 150 μL/well of logarithmic growth phase of RAW264.7 cells suspension was placed in a 96-well culture plate at a density of 1.0×10^5 cells/mL and incubated for 24 h (37°C, 5% CO_2). Then 100 μL of FMP2-1 at different concentrations (20 μg/mL, 50 μg/mL, 100 μg/mL, 200 μg/mL and 500 μg/mL) and LPS (for the control group) were added into the 96-well culture plate. After incubation, 20 μL of the MTT solution (5 mg/mL) was added to each well and the mixture was further incubated for 4 h. After the supernatant was removed, 200 μL of DMSO was added to dissolve the dye crystal and the plate was shaken for 10 min. The optical density was measured at 570 nm using a multifunctional microplate reader (Multiskan Go 1510, Thermo Fisher Scientific). The absorbance was translated into macrophage proliferation index (MPI) by the following formula:

$$\mathrm{MPI} = \frac{A_1}{A_0}$$

where A_1 and A_0 are the absorbance of the sample group and control group, respectively.

Measurement of NO production: The nitric oxide (NO) production in the RAW264.7 cells was assayed by Griess reaction. 150 μL/well of logarithmic growth phase of RAW264.7 cells suspension was placed in a 96-well culture plate at a density of 1.0×10^5 cells/mL and incubated for 24 h (37°C, 5% CO_2). Then 100 μL of FMP2-1 at different concentrations (20 μg/mL, 50 μg/mL, 100 μg/mL, 200 μg/mL and 500 μg/mL) and LPS (for the control group) were added into the 96-well culture plate. After incubated at 37°C for 24 h, the culture supernatant (100 μL) and an equal volume of Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% N-[1-naphthyl]-ethylenediamine dihydrochloride in distilled water) were mixed in 96-well plates and incubated with gentle shaking for 10 min. The absorbance was measured at 550 nm in a multifunctional microplate reader. $NaNO_2$ was used as a standard.

Measurement of phagocytosis: 150 μL/well of logarithmic growth phase of RAW264.7 cells suspension was placed in a 96-well culture plate at a density of 1.0×10^5 cells/mL and incubated for 24 h (37°C , 5% CO_2). Then 100 μL of FMP2-1 at different concentrations (20 μg/mL, 50 μg/mL, 100 μg/mL, 200 μg/mL and 500 μg/mL) and LPS (for the control group) were added into the 96-well culture plate. After incubated at 37°C for 48 h, the culture supernatant was removed and the adherent cells were washed with PBS buffer two time. 150 μL of 0.1% neutral red was added to each well. After incubated at 37°C for 4 h, the residual neutral red was removed by PBS. 100 μL of cell lysate (ethanol and acetic acid mixed at same volume) was added to each well and the plate was kept for 2 h. The absorbance was measured at 550 nm in a multifunctional microplate reader.

Statistical analysis: All experiments were conducted in three replicates. Data were expressed as means ± standard deviations (SD). The results were analyzed by commercially available statistical software.

RESULT AND DISCUSSION

Structural Characterization of FMP2-1

Preparation and basic properties: Crude polysaccharides (FMP) were isolated from the fruiting bodies of floral mushroom by the method described for polysaccharides. One homogeneous polysaccharide fraction, termed FMP2-1, was purified by DEAE-52 anion exchange chromatography and Sephacryl S-300. The sugar content of FMP2-1 was 97.54%. In addition, FMP2-1was free of protein and uronic acid, as evidenced by a negative response to the Coomassie Brilliant Blue G-250 method and *m*-hydroxydiphenyl method. FMP2-1 showed one symmetrical and single peak in the HPGPC (Figure 1a), suggesting it was homogeneous fraction. According to the calibration curve with standard dextrans, the average molecular weight of FMP2-1 was calculated as 8.4×10^6 Da. FT-IR spectroscopy was used to confirm the characteristic organic groups in molecules [26]. The FT-IR spectrum of FMP2-1 was presented in Figure 1b. The strong and broad band around 3410 cm^{-1} was assigned to O-H stretching vibration of polysaccharide. The absorption peak at 2920 cm^{-1} was ascribed to C-H asymmetric vibration and the absorption at 1045 cm^{-1} was attributed to the existence of C-O bond in polysaccharide. The weak absorbance at 1415 cm^{-1} represented the O-H bending vibration. The absorption bands at 850 cm^{-1} for the polysaccharide suggested the presence of α configuration (Figure 1c) [27].

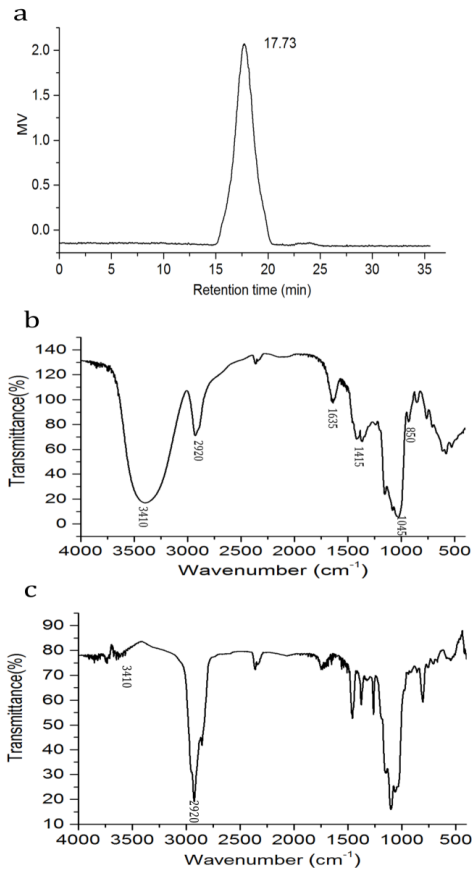

Figure 1: (a) HPGPC chromatogram of FMP2-1, (b) FT-IR spectrum of FMP2-1, (c) FT-IR spectrum of the methylated polysaccharide

Monosaccharide composition: After the FMP2-1 fraction was completely hydrolyzed, reduced, acetylated, converted into alditol acetates in the end, the resulting product was analyzed by GC (Figure 2). The monosaccharide composition of FMP2-1 was identified by retention times with standard monosaccharides. The result indicated that the FMP2-1was composed of glucose.

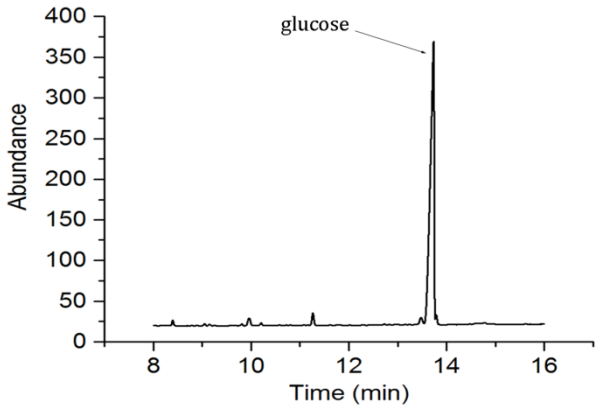

Figure 2: Monosaccharide composition analysis of FMP2-1

Methylation analysis: To determine the glycosidic linkages of monosaccharide, FMP2-1 was fully methylated, acid hydrolyzed, acetylated and then converted into alditol acetates for the GC-MS analysis. The methylation of FMP2-1 was confirmed by the FT-IR analysis (Figure 1c). Compared Figure 1c to Figure 1b, it showed that the absence of peak at 3410 cm^{-1} and the enhancement of absorption peak at 2920 cm^{-1}, which demonstrated that the methylation was completed. The results of GC-MS analysis were shown in Table 1. FMP2-1 showed four derivatives, namely 2,3,4,6-Me4-Glcp, 2,3,6-Me3-Glcp, 2,3,4-Me3-Glcp, 2,3-Me2-Glcp. The peak areas under the curve represent the molar ratios. The molar ratio was 1.00 : 13.09 : 15.01 : 0.97, respectively. According to the methylation analysis, the monosaccharide composition was consistent with the result by GC-MC analysis.

Table 1: GC-MS date for methylation analysis of FMP2-1 from floral mushroom

Methylated sugars	Linkages types	Molar ratios	Mass fragments (m/z)
2,3,4,6-Me4-Glcp	T-Glcp	1.00	45,71,87,101,117,129,145,161,205
2,3,6-Me3-Glcp	1,4-Linked- Glcp	13.09	45,71, 87,99,101,113,129,143,161,233
2,3,4-Me3-Glcp	1,6-Linked- Glcp	15.01	45,71,87,99,101,129, 161,189,233
2, 3-Me2-Glcp	1,4, 6-Linked- Glcp	0.97	43,45,89,99, 101,117,142,159, 201, 261

NMR analysis: Structural properties of FMP2-1 were further investigated by NMR spectroscopy. The NMR spectra of polysaccharide were displayed in Figure 3. According to the previous studies [28,29], the ^{1}H NMR signals at δ 5.36 *ppm*, 5.19 *ppm*, 4.61 *ppm* and 4.49 *ppm* were assigned to the anomeric proton of 1,4-Linked-Glc*p*, 1-Linked-Glc*p*, 1,4,6-Linked-Glc*p* and 1, 6-Linked-Glc*p*. The peaks at 103.1 *ppm*, 99.6 *ppm*, 95.8 *ppm* and 92.0 *ppm* in the ^{13}C NMR spectrum were ascribed to the anomeric carbon of 1, 6-Linked-Glc*p*, 1, 4-Linked-Glc*p*, 1, 4, 6-Linked-Glc*p* and 1-Linked-Glc*p*, respectively [30]. In the ^{13}C spectrum, the signals resulting from α-anomeric carbons typically exist in the range 95–101 *ppm* while majority of the β-anomeric carbons will emerge in the 101–105 *ppm* region. According to the chemical shifts of anomeric carbons, both α and β anomeric configurations for monosaccharide residues were exist in FMP2-1. Other signals of chemical shifts for monosaccharide residues were assigned and shown in Table 2. In HSQC, the cross peaks of anomeric proton and anomeric carbon signals at A (5.36/99.6), B (5.19/92.0), C (4.61/95.8) and D (4.49/103.1) were assigned. The connectivity of the glycosyl residues in FMP2-1 was confirmed by HMBC spectrum. Two clear cross peaks were detected between the H-1 (δ 5.36 *ppm*) of residue A to C-4 (δ 76.7 *ppm*) of residue A (A H1/A C4) and H-4 (δ 3.60 *ppm*) of residue A to C-1 (δ 99.6 *ppm*) of residue A (A H4/A C1) suggesting that the C-1 of residue A was linked to O-4 position of residue A. Similarly, the strong cross peaks of D H1/D C6 at 4.49/68.7 *ppm* (D H1/D C6) and D H6/D C1 at 3.81/103.1 *ppm* (D H6/D C1) were demonstrated that the C-1 of residue D was linked to O-6 position of residue D. The inter-residue HMBC correlations from H-1 of residue A to C-4 of residue C and H-4 of residue C to C-1 of residue A indicated the linkage of C-1 of residue A to the O-4 position of residue C. Those were corresponding to the results of GC-MS analysis. On the basis of methylation analysis and HMBC spectrum of FMP2-1 demonstrated that (1→4)-α-D-Glc*p* and (1→6)-β-D-Glc*p* were the main linkage manner. Furthermore, the linkage of residues C-1 of residue B to the O-6 position of residue C was indicated by the cross peak at 5.19/69.9 *ppm* (B H1/C C6).

Based on the monosaccharide composition analysis, methylation analysis and NMR analysis, it was concluded that FMP2-1 was a glucan consisting of a (1→4)-α-D-Glc*p* and (1→6)-β-D-Glc*p* with the branched chain T-β-D-Glc*p* substituted at O-6 position of (1→4)-α-D-Glc*p*.

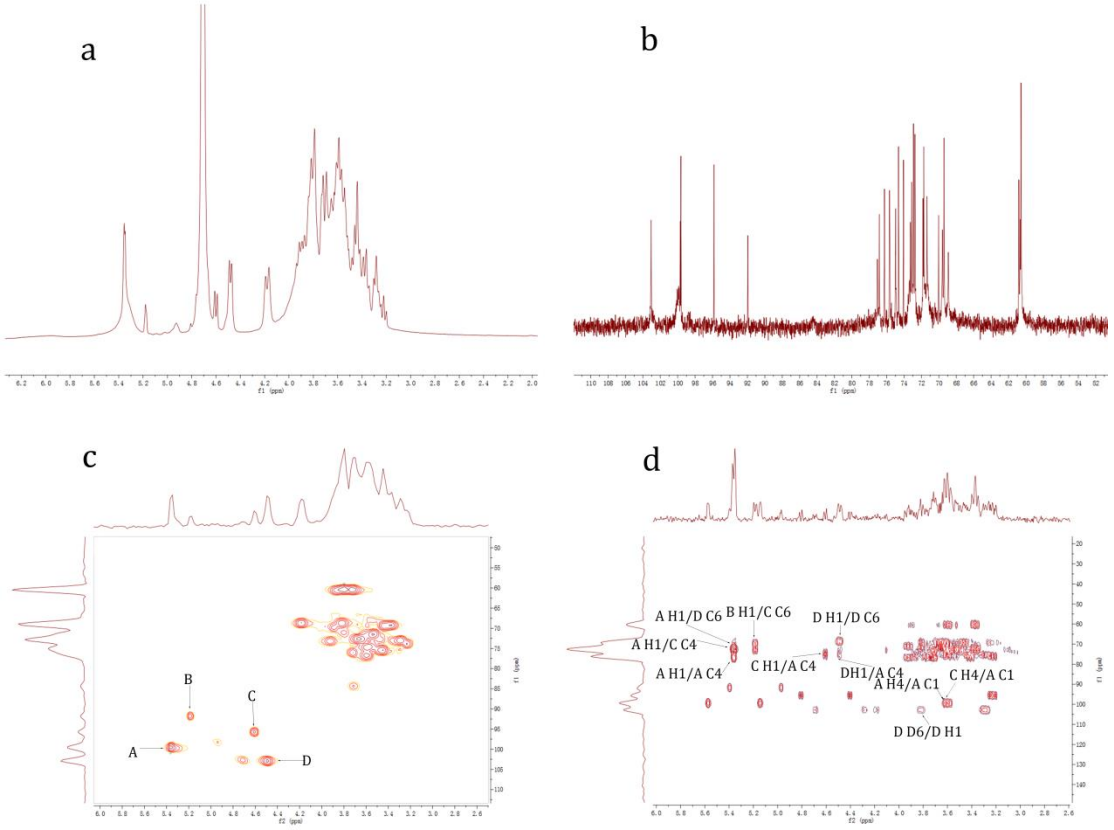

Figure 3: (a) ^{1}H NMR spectrum of FMP2-1 in D_2O, (b) ^{13}C NMR spectrum of FMP2-1 in D_2O, (c) HSQC NMR spectrum of FMP2-1 in D_2O and (d) HMBC NMR spectrum of FMP2-1 in D_2O

Table 2: Chemical shifts of resonances in ^{1}H NMR and ^{13}C NMR of FMP2-1 from floral mushroom

Glycosyl residues	H-1/C-1	H-2/C-2	H-3/C-3	H-4/C-4	H-5/C-5	H-6/C-6
A →4)-α-D-Glc*p*-(1→	5.36 99.6	3.69 71.8	3.47 77.3	3.60 76.7	3.55 71.7	3.74 60.6
B α-D-Glc*p*-(1→	5.19 92.0	3.23 74.1	3.84 85.2	3.43 69.4	3.59 74.6	3.80 60.7
C →4,6)-α-D-Glc*p*-(1→	4.61 95.8	3.35 71.6	3.63 79.1	3.59 76.9	3.58 71.6	3.89 69.9
D →6)-β-D-Glc*p*-(1→	4.49 103.1	3.30 75.6	3.73 76.1	3.89 70.1	3.69 71.8	3.81 68.7

Immunomodulatory activity of FMP2-1: Macrophages, one kind of immune cells, exist in almost all physiological organizations of the body and was an important medium to study the immune functions of polysaccharides [31]. Partial acid hydrolysis was commonly performed to elucidate the effect of branching and average molecular weigh on immunologic activity. The molecular weight of partial acid hydrolysis product of FMP2-1 (FMP2-1S) determined through HPGPC was 6.9×10^6 Da which was smaller than FMP2-1. In order to reveal the potential immunemodulatory of polysaccharides from floral mushroom, FMP2-1 and FMP2-1S were used to study macrophage proliferation, NO production and phagocytic activity.

Cell toxicity of FMP2-1 to macrophages: The effect of FMP2-1 and FMP2-1S on RAW264.7 cells proliferation was detected by MTT assay in the dose range of 20–500 μg/mL, as shown in Figure 4a. Both FMP2-1 and FMP2-1S could stimulate macrophage RAW264.7 cells proliferation compared with that of LPS. FMP2-1 under 200 μg/mL displayed a dose-dependent proliferative effect. The values of proliferation index increased with increased polysaccharide concentrations. The maximum proliferation index was 1.72 at the concentration of 200 μg/mL. At the higher concentration ranges of 200–500 μg /mL, proliferation index decreased with increased concentrations. The effect of FMP2-1S on RAW264.7 cells was similar to that of FMP2-1. The proliferation indexes of FMP2-1S were 1.25, 1.25, 1.30, 1.34 and 1.31 respectively. Upon comparing with FMP2-1, the lower reducing proliferation index of FMP2-1S could be observed. Therefore, the deletion of branching and reduction of Mw caused by partial acid hydrolysis was demonstrated to weaken the proliferation activity of FMP2-1.

NO secretion from macrophages: It is well known that NO is an important cytokine from RAW264.7 and play pivotal roles in immunomodulatory activities. Treatment of RAW264.7 cells with polysaccharide fractions resulted in a clearly increased effect in the production of NO (Figure 4b). As show in Figure 4b, the result indicated that FMP2-1 and FMP2-1S were effective in stimulating NO production by RAW264.7 cells. The NO production of FMP2-1 increased from 6.92 to 10.39 as the polysaccharide concentration increased from 20 μg/m to 100 μg/mL. Compared with control group, the production of NO increased significantly after treatment with 100 μg/mL of FMP2-1 and reached 10.39 μmol/L. However, the level of NO decreased with increased FMP2-1 concentrations when the concentrations were more than 100 μg/mL. Similarly, the group of FMP2-1S has a parallel growth trend in stimulating macrophages RAW264.7 to produce NO. When compared with the control group, the NO production was significantly enhanced and the highest level was expressed at 100 μg/mL, which displayed a 2.6-fold increment compared to the control. Overall, the NO production of the FMP2-1S group was enhanced. This allowed to conclude that the deletion of branching and reduction of Mw strengthen the NO production of FMP2-1. The effect of FMP2-1 on NO production indicated that FMP2-1 effectively activated RAW264.7 cells.

Macrophage phagocytosis: The phagocytic activity of activated macrophages was one of the defense mechanisms in the immune response and an item to size the immunity of organism [32,33]. As shown in Figure 4c, two fractions had similar enhancing effects on the phagocytic activity of RAW264.7 cells in the dose range of 20–500 μg/mL and the optimum dose for them all was 200 μg/mL. Furthermore, the activity of the two polysaccharides was weaker than LPS at all concentrations. FMP2-1S had stronger macrophage phagocytosis activity probably due to the removal of branching and reduction of Mw. No significant differences between FMP2-1 and FMP2-1S were observed in polysaccharides-treated group. These results suggested that FMP2-1 could stimulate macrophages for enhancing phagocytosis capacity.

Combined with above results, we confirmed that the potential of FMP2-1 as an immunomodulatory agent by increasing the proliferation, NO production and phagocytic activity in RAW264.7 cells.

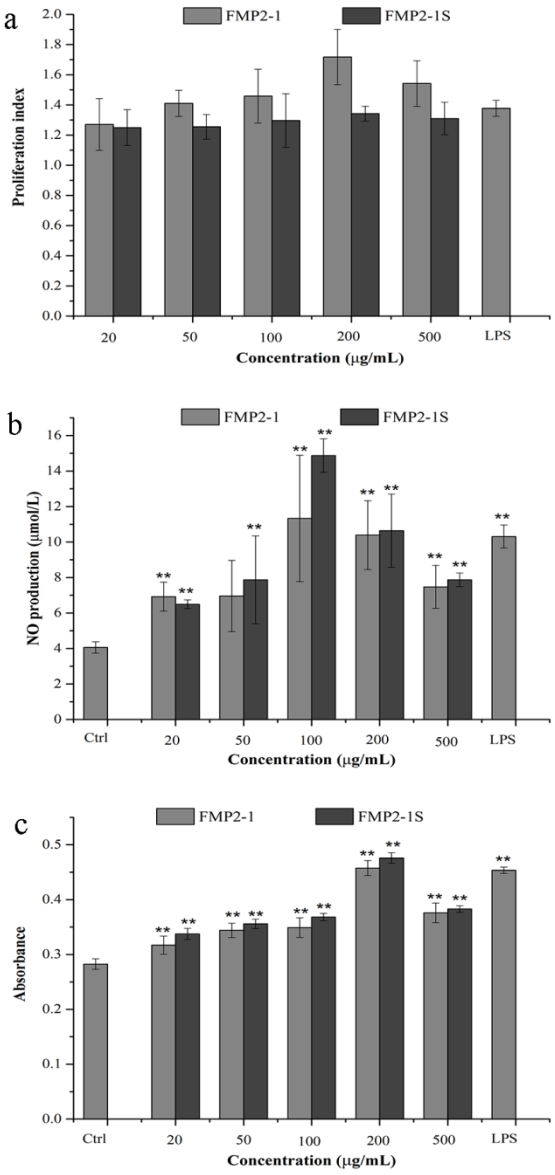

Figure 4: (a) Effect of FMP2-1 on the cell proliferation, (b) NO production and (c) phagocytosis activity of RAW264.7 macrophages. All experiments were repeated at least three times

CONCLUSION

In conclusion, a homogeneous polysaccharide FMP2-1 was extracted and purified from the fruit bodies of floral mushroom. FMP2-1 consisted of glucose with an average molecular weight of 8.4×10^6 Da. The backbone was constructed by (1→4)-α-D-Glc*p* and (1→6)-β-D-Glc*p*. The branch chains T-β-D-Glc*p* substituted at O-6 position of (1→4)-α-D-Glc*p*. The immunoregulatory activity experiments showed that FMP2-1 could promote the proliferation and NO production of macrophage RAW264.7 and enhance the phagocytosis. The lack of branched chain and decrease of Mw could reduce those effects. Consequently, above results suggest the potential of FMP2-1 as natural immunomodulatory agent.

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