Carbohydrate Polymers 80 (2010) 783–789

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Extraction, purification, characterization and antitumor activity of polysaccharides from *Ganoderma lucidum*

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ARTICLE INFO

Article history:
Received 3 November 2009
Received in revised form 11 December 2009
Accepted 15 December 2009
Available online 4 January 2010

Keywords:
Ganoderma lucidum polysaccharides
Purification
Characterization
Antitumor activity

ABSTRACT

Ultrasonic-aid extraction (UAE) was applied to the extraction of polysaccharides from *Ganoderma lucidum* and then the crude polysaccharides were purified by filtration, DEAE cellulose-52 chromatography and Sephadex G-100 size-exclusion chromatography in that order. Two main fractions, GP-1 and GP-2, were obtained through the extraction and purification steps. The characterizations, such as molecular weight, monosaccharides composition, ultraviolet spectrum and infrared spectrum of the two fractions were analyzed in this study. Furthermore, the influence of *G. lucidum* polysaccharides fractions upon activation of macrophage cell (RAW 264.7) and antitumor activities to the human breast cancer cell (MDA-MB-231) in vitro were evaluated by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay. The results indicated that GP-1 and GP-2 can increase the proliferation and pinocytic activity of macrophage significantly and play an inhibited effect on the cancer cell, moreover, the antitumor activity of the GP-1 and GP-2 increased with the participation of the antitumor factors induced from macrophage by polysaccharides fractions.

1. Introduction

For thousands of years, mushrooms have been known as a source of medicine. *Ganoderma lucidum*, which belongs to the family of *Ganodermataceae* of *Polyporales*, is known as 'Lingzhi'. In East Asia, the fruiting body of *G. lucidum* has been used for centuries and it is also called 'marvelous herb'. It has been reported that *G. lucidum* polysaccharides have strong antioxidant activities (Chen et al., 2009; Jia et al., 2009; Xu et al., 2009), immuno-modulating activities (Lin et al., 2006; Shao, Dai, Xu, Lin, & Gao, 2004), and antitumor activities (Li, Fang, & Zhang, 2007; Paterson, 2006; Yuen & Gohel, 2008; Zhang, Cui, Cheung, & Wang, 2007), etc.

Extraction is widely used for the separation of these biologically functional components from various plant, bacteria, or animal sources. The processes of conventional liquid extraction such as stirring extraction and Soxhlet extraction for solid and semisolid materials are generally time-consuming and laborious. Ultrasound-assisted extraction (UAE) is an environmentally friendly technique with increased productivity. It uses high-frequency (typically higher than 16 kHz) sound to disrupt the target compound from cells (Raso & Barbosa-Canovas, 2003). To date, there have been several reports on the application of UAE in the separation of various biologically active compounds such as the anti-cancer drug camptothecin from *Nothapodytes foetida* (Fulzele & Satdive, 2005), isoflavones from freeze-dried ground soybean (Rostagno, Palma, & Barroso, 2003), phenolic compounds from alperujo (Priego-Capote, Ruiz-Jimenez, & de Castro, 2004), and astaxanthin from microorganisms (Han, Lee, Jung, & Choi, 2002). But there is hardly any report that UAE is applied to separate the polysaccharides from *G. lucidum*.

In this study, we isolated polysaccharides from *G. lucidum* by UAE, filtration, DEAE cellulose-52 chromatography and size-exclusion chromatography in order, analyzed the elementary characterization of the polysaccharides fractions, and evaluated the influence of *G. lucidum* polysaccharides fractions upon activation to macrophage cell (RAW 264.7) and antitumor activities to the human breast cancer cell (MDA-MB-231) in vitro.

2. Materials and methods

2.1. Materials and reagents

Fruiting bodies of *G. lucidum* were obtained from Jiangsu Alphay Biological Technology Co., Ltd. (Nantong, China). The human breast cancer cell MDA (MDA-MB-231) was obtained from Jiangsu Academy of Agricultural Sciences (Nanjing, China). Raw murine macrophage (RAW 264.7) was purchased from ATCC (USA). The following chemicals were used: DEAE-52 and Sephadex G-100 were purchased from Whatman Co. (Maidstone, Kent, UK) and Pharmacia

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doi:10.1016/j.carbpol.2009.12.029

Co. (Sweden), respectively. Rhamnose, arabinose, xylose, mannose, glucose, galactose, fucose, inositol, dimethylsulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). RPMI 1640 medium was purchased from GIBCO Co. (USA). Fetal bovine serum (FBS) was obtained from Hangzhou Sijiqing Biological Engineering Materials Co., Ltd. (Hangzhou, China). Penicillin and streptomycin were purchased from Invitrogen (Carlsbad, CA, USA). All other reagents were of analytical grade.

2.2. Preparation of G. lucidum polysaccharides

The samples were dried at 50 °C, cut into small pieces and extracted with 95% ethanol for 24 h to remove impurities and small lipophilic molecules. The degreased powders (10 g) were dried, and extracted with distilled water (280 ml) by ultrasonic waves with a JY98-cell-breaking apparatus prior (Scientz Biotechnology Co., Ningbo, China) for 17 min. The aqueous extract was centrifuged at 4500 rpm for 20 min to remove the pellet, concentrated and the supernatant precipitated by addition of a 4-fold volume of anhydrous ethanol and then incubated at 4 °C for 24 h. After centrifugation, the precipitate was washed with anhydrous ethanol, acetone and ether in turn, and then dried to yield the crude polysaccharides.

The crude polysaccharide was re-dissolved in 50 ml distilled water, filtered through 4.5×10^{-4} mm filters and applied to a DEAE-52 cellulose column (2.6×30 cm) equilibrated with distilled water. The polysaccharide was fractionated and eluted with distilled water and different concentrations of stepwise NaCl solution (0, 0.1 and 1 M NaCl). The elutes were concentrated to obtain the main fractions, which were then fractionated by size-exclusion chromatography on a Sephadex G-100 column (2.6×60 cm) eluted with 0.05 M NaCl at a flow rate of 0.5 ml/min. The fractions obtained were combined according to the total carbohydrate content quantified by the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The relevant fractions were collected, concentrated, dialyzed and lyophilized. For analysis of biological activity, the fractions were diluted in DMEM culture medium to a concentration of 2 mg/ml and filtered through sterile 0.22 μm filters.

2.3. Characterization of polysaccharide fractions

2.3.1. Ultraviolet analysis

Two fractions were dissolved and diluted to 2 mg/ml respectively, and the solutions of the polysaccharide fractions were scanned from 200 to 400 nm with a 722 spectrophotometer (Shanghai Precision and Scientific Instrument Co., Ltd.).

2.3.2. Homogeneity and molecular weight of polysaccharide fractions

The homogeneity and average molecular weight of the polysaccharide fractions were determined by HPGFC using a Waters 600 HPLC and Ultrahydrogel™Linear column (300×7.8 mm) eluted with 0.1 M $NaNO_3$ at a flow rate of 0.9 ml/min. Peaks were detected using a 2410 differential refractive index detector. The molecular weights of the polysaccharide fractions were determined by comparison with retention times of pullulan standards (MW 2000000, 133800, 41100, 21400, 4600 and 180).

2.3.3. Infrared analysis

IR spectra were recorded using the KBr-disk method with a Nicolet Fourier transform infrared (FTIR) spectrometer (NICOLET NEXUS470, Spectrum One, Thermo Nicolet Co., Madison, WI, USA) in the range 400–4000 cm^{-1}.

2.3.4. Monosaccharides composition

One-hundred μl of polysaccharide fractions, with the concentration 4–5 g/L, were hydrolyzed with 100 μl 4 mol/LTFA at 110 °C for 2 h. After removing TFA with methanol, samples were dissolved in NaOH and pre-column derivated with 1-phenyl-3-methyl-5-pyrazolone (PMP) using the method described by Dai, Zhu, Tang, Wang, and Chen (2007). The PMP derivatives of the ten standard sugars (Man, Rib, Rham, GlcUA, GalUA, Glc, Gal, Xyl, Ara, Fuc) and *G. lucidum* polysaccharides were subjected to Agilent1100 High Performance Liquid Chromatography (Dionex Co., America), fitted with EclipseXPB-C18 Column (4.6 mm × 250 mm).

2.4. Cell culture

Raw murine macrophage (RAW 264.7) and human breast cancer cell MDA (MDA-MB-231) were grown in DMEM with phenol red and supplemented with 10% (v/v) bovine serum, 100 U/ml streptomycin and penicillin at 37 °C in a humidified atmosphere containing 5% CO_2. Cells were grown to confluence in sterile tissue culture flasks and gently detached by pancreatic enzyme.

2.5. Pinocytic activity assay

The pinocytic activity was determined according to the method of Cheng, Wan, Wang, Jin, and Xu (2008). Macrophages were suspended in DMEM containing various concentrations of polysaccharide fractions (0, 5, 25 and 50 μg/ml). Cells were placed in a 96-well plate and cultured at 37 °C, 5% CO_2 for 24 h. Culture media were removed, 100 μl/well 0.075% neutral red was added, and incubated for another 3 h. Media were discarded, and cells were washed twice with PBS (pH 7.2–7.4). Then 150 μl/well of cell-lysis buffer was added and cultured for 4 h. The absorbance at 490 nm was measured using an ELISA reader.

2.6. Proliferation assay

The MTT assay (Taira, Nanbu, & Ueda, 2009) was used to examine the cell viability due to treatment with polysaccharide fractions, which is based on the ability of mitochondrial enzyme, succinate dehydrogenase to cleave MTT to the blue compound formazan. Briefly, the cells were seeded at a density of 5.0×10^5 cells/ml and permitted to adhere for 12 h. After the culture medium was removed, 100 μl of different concentrations of polysaccharides (0, 5, 25 and 50 μg/ml), prepared in DMEM, were added to each well. After 24 h of exposure, 10 μl of a 5 mg/ml solution of MTT was added to each well and incubated for 4 h and the suspension was removed. Extraction with DMSO (100 μl) was measured at 490 nm, using a microplate reader (BIO-RAD Model 550, BIO-RAD, USA).

$$\text{Activation rate}\,(\%) = (\text{Absorbance of experimental group} - 1/\text{Absorbance of blank control group}) \times 100\%.$$

2.7. Preparation of polysaccharide and RAW 264.7 cell co-culture media

RAW 264.7 cells were grown in phenol red DMEM containing 10%FBS. 500 μl cells were plated at a density of 5×10^5 cells per well in 6-well plates. After a pre-incubation period of 12 h, the medium was removed. Cells, suspending in 500 μl DMEM containing various concentrations of both fractions GP-1 and GP-2 (0, 5, 25 and 50 μg/ml), were incubated for 24 h. The co-cultured medium, named GP1-MΦ-CM, GP2-MΦ-CM and N-MΦ-CM (control) were collected, filtered through a Millipore membrane filter with an average pore diameter of 0.22 μm. The medium was stored at −20 °C for future research.

2.8. Growth inhibition of antitumor cell

2.8.1. Inhibition effect on antitumor cell of polysaccharide medium

The adhered cells were treated with 100 μl DMEM containing various concentrations of both fractions GP-1 and GP-2 (0, 5, 25 and 50 μg/ml) and incubated for 24 h. The inhibition effects of polysaccharide fractions on the growth of MDA cells were evaluated in vitro by MTT assay as described in Section 2.7. The inhibition rate was calculated according to the formula below:

$$\text{Growth inhibition rate } (\%) = (1 - \text{Absorbance of experimental group}/\text{Absorbance of blank control group}) \times 100\%.$$

2.8.2. Inhibition effect on antitumor cell of GP1-MΦ-CM and GP2-MΦ-CM

The adhered cells were treated with 100 μL GP1-MΦ-CM, GP2-MΦ-CM and N-MΦ-CM and incubated for 24 h. The inhibition effects of polysaccharide fractions on the growths of MDA cells were evaluated in vitro by MTT assay as described in Section 2.7.

2.9. Statistical analysis

The data were presented as means ± SD of three determinations. Statistical analyses were performed using student's *t*-test and one way analysis of variance. Multiple comparisons of means were done by the least significance difference test. All computations were done by employing the statistical software (SAS, version 8.0).

3. Results and discussion

3.1. Extraction and purification of water-soluble polysaccharide

Crude polysaccharide was isolated from *G. lucidum* and the yield was about 2.07%. Neutral and acidic polysaccharide was obtained from *G. lucidum*. The extracts were fractionated by preparative size-exclusion chromatography to obtain two main fractions, which were selected based on total carbohydrate elution profile. The neutral polysaccharide, designated as GP-1, was eluted by distilled water, while the acidic polysaccharide GP-2 was then eluted at a higher salt concentration (Fig. 1A). The two fractions were subjected to gel filtration on a Sephadex G-100 column. As seen from Fig. 1B, GP-1, yielded a single peak, which reached the peak absorbance at tube 34, same as GP-2 which reached the peak absorbance at tube 18 (Fig. 1C).

3.2. Characterization of polysaccharide fractions

Both polysaccharide fractions had no absorption at 280 and 260 nm in the UV spectrum (Fig. 2), indicating the absence of protein and nucleic acid.

High performance gel filtration chromatography (HPGFC) of the individual fractions showed that each fraction was represented by a broad, symmetrical peak on the chromatograms (Fig. 3A and B). The average molecular weights of the two fractions were determined to be 1.926 and 1086 kDa for fractions GP-1 and GP-2, respectively.

Analysis of sugar composition of the polysaccharide fractions indicated that both polysaccharides consisted primarily of glucose

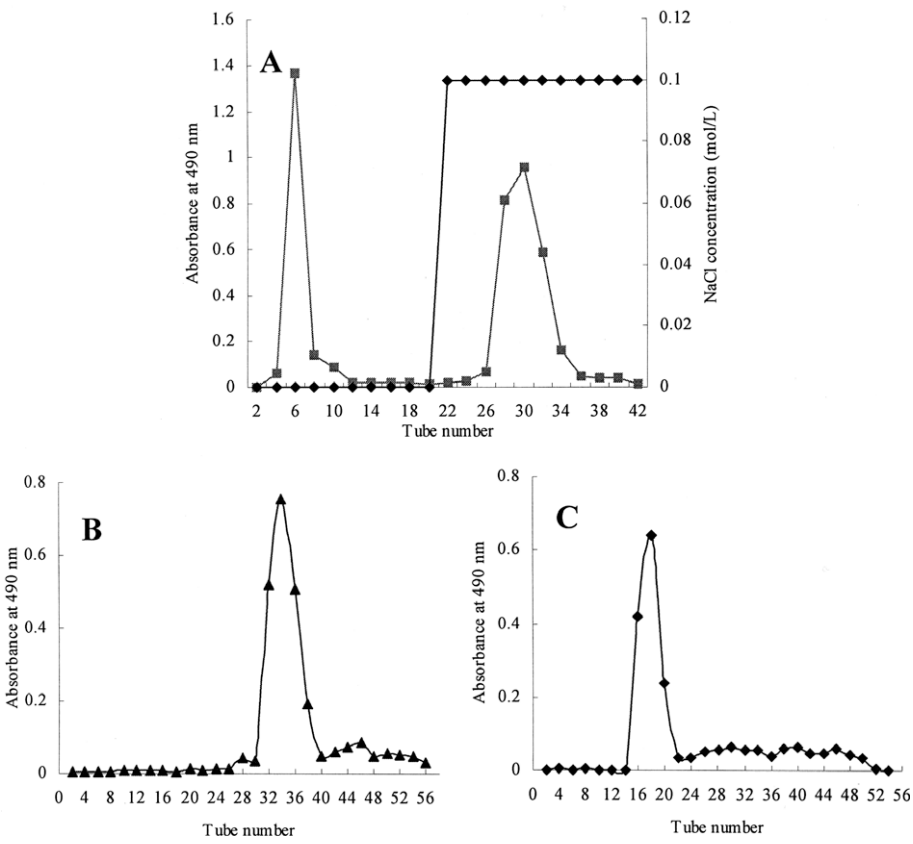

Fig. 1. Fractionation of Artemisia polysaccharides by size-exclusion chromatography. (Panel A) Polysaccharides from crude Artemisia extract were isolated by DEAE-cellulose-52 chromatography, named as GP1 and GP2. The eluate was further fractionated using Sephadex G-100 column chromatography (Panel B and Panel C). Total carbohydrate content of the fractions was determined by a phenol–sulfuric acid method (detected at 490 nm). (Panel B) GP1. (Panel C) GP2. –◇–, ion concentration; –□–, content of carbohydrate.

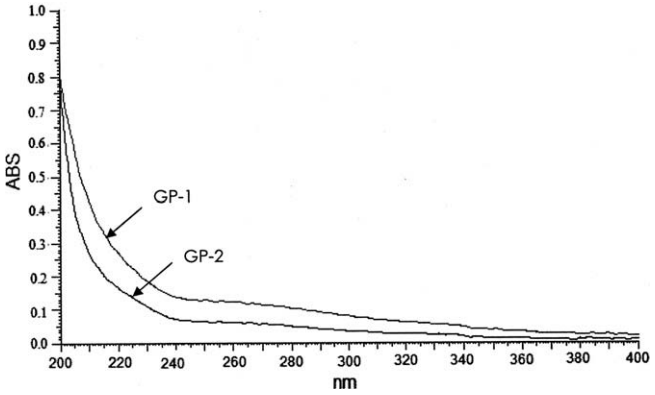

Fig. 2. The UV spectra of polysaccharide fractions of *Ganoderma lucidum*.

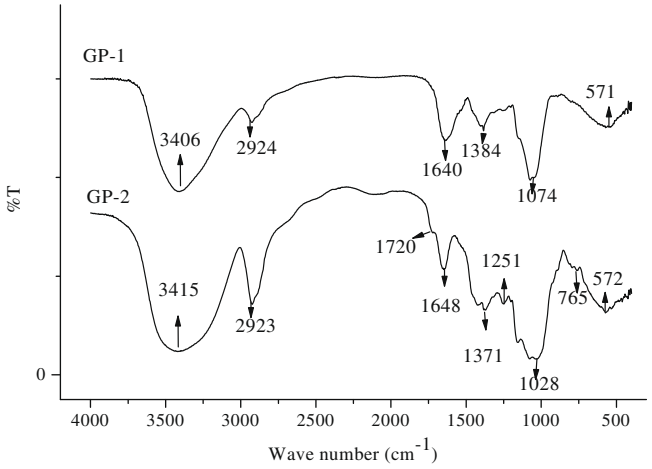

Fig. 4. The IR spectra of polysaccharide fractions of *Ganoderma lucidum*.

and galactose, which accounted for the majority of monosaccharides present (Table 1). Small amounts of mannose, rhamnose and fucose were detected in both the fractions, with GP-2 also consisted of a minor amount of glucuronic acid. The sugar composition of these fractions are similar to monosaccharides compositions of PSG-1 from *Ganoderma atrum* by Chen, Xie, Nie, Li, and Wang (2008).

Both infrared spectrum of the purified GP-1 and GP-2, as shown in Fig. 4, displayed a broadly-stretched intense peak at 3400 cm^{-1} characteristic of hydroxyl groups and a weak C—H band at around 2920 cm^{-1}. The relatively strong absorption peak at around 1640 cm^{-1} indicated the characteristic of C=O (Ge, Duan, Fang, & Wang, 2009). The absorbance of polysaccharides in the range 950–1200 cm^{-1} were where the C—O—C and C—O—H link band positions were found (Kacurakova, Capek, Sasinkova, Wellner, & Ebringerova, 2000). The bands in the range of 350–600 cm^{-1} are assigned to skeletal modes of pyranose rings (Yang & Zhang, 2009). The band towards 1720 and 1251 cm^{-1} of GP-2 indicated the trace of uronic acids (Chen et al., 2008) and ester sulfate in the samples (Melo, Feitosa, Freitas, & de Paulaet, 2002).

3.3. Effect of G. lucidum polysaccharides on macrophage cells proliferation

Polysaccharide is an activator extracted from *G. lucidum*, and can effectively activate cell proliferation. As seen in Table 2, there was significant increase in cell proliferation rate when RAW 264.7 macrophages were incubated with different concentrations of both fractions. Treatment of these cells with GP-1 resulted in a dose-dependent increase in cell proliferation rate with a highest rate of 150.39% at 50 μg/ml. In comparison, at higher concentrations (50 μg/ml) polysaccharide GP-2 actually decreased macrophage proliferation rate. Furthermore, the rate of cell proliferation at 5–50 μg/ml concentrations of GP-2 was comparable to or even greater than that elicited by 50 μg/ml GP-1.

Ganoderma lucidum (*G. lucidum*) is a popular medicinal mushroom that has been used as a home remedy for the general promotion of health and longevity in East Asia. Stanley, Harvey, Slivova, Jiang, and Sliva (2005) and Xie et al. (2006) have previously dem-

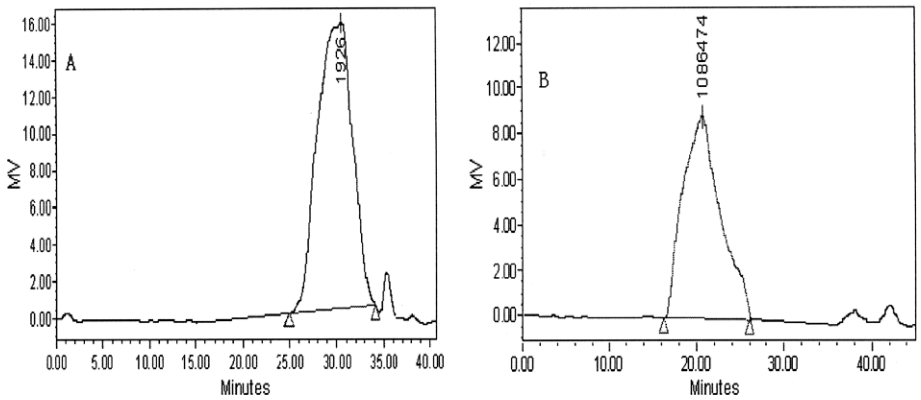

Fig. 3. Fractions GP-1 and GP-2 were analyzed by HPGFC and monitored with a differential refractive index detector, as described. (Panel A) GP1. (Panel B) GP2.

Table 1
Sugar composition of polysaccharide fractions.

Samples	Molar rations (mol%)						
	Mannose	Ribose	Rhamnose	Glucuronic acid	Glucose	Galactose	Fucose
GP-1	3.10	nd	0.53	nd	60.11	30.58	5.67
GP-2	9.89	nd	0.35	1.46	68.04	15.81	4.45

The data are presented as mol% for each sugar. Individual components were identified and quantified based on elution of known standards. nd, not detected.

Table 2
Activation of RAW 264.7 exposed to different concentrations of *Ganoderma lucidum* polysaccharides. Cells were incubated for 24 h with the indicated concentrations of polysaccharide fractions. Values are mean ± SD of four replicates. Control cells were incubated with medium alone. LPS was the positive control.

Treatment	Concentration (μg/ml)	OD (490 nm)	Proliferation rate (%)
Control	–	0.462 ± 0.006	100.00
LPS	2	0.892 ± 0.031	193.07
GP-1	50	0.695 ± 0.002**,a	150.39
	25	0.620 ± 0.071**,a	134.20
	5	0.614 ± 0.011**,a	132.90
GP-2	50	0.713 ± 0.018**,a	154.22
	25	0.746 ± 0.073**,a	161.53
	5	0.714 ± 0.029**,a	154.55

** $p < 0.01$, significantly different from the control.
[a] $p < 0.01$, significantly different from LPS-treated cells as positive control.

onstrated that *G. lucidum* and *G. lucidum* polysaccharide have the inhibition of cancer cell proliferation. However, the mechanism(s) responsible for the inhibitory effects of *G. lucidum* on the cancer cells has not been fully elucidated. In this study, the immunity activity of *G. lucidum* polysaccharide was examined at the cellular level, which helped explain the mechanism of inhibitory effects of *G. lucidum* polysaccharide on the breast cancer cells.

As the first step towards understanding the immunomodulatory activity of polysaccharide, the effect of GP-1 and GP-2 on macrophage cells proliferation was investigated. As seen from the Table 2, both two fractions had promoted farthest proliferation of macrophages. Especially, at the concentration of 25 μg/ml, proliferation activity of GP-2 on macrophages was up to maximum. Some results have also found the similar effect of other mushroom polysaccharide in animal models. Likewise, intravenous injection of lentinan increased the absolute number of monocytes in peripheral blood, as well as number of granulocyte–macrophage progenitor cells in spleen and bone marrow (Schepetkin & Quinn, 2006).

3.4. Effect of G. lucidum polysaccharides on pinocytic activity in macrophages

One of the most distinguished features of macrophage activation would be an increase in pinocytic activity (Cheng et al., 2008). To further investigate whether *G. lucidum* polysaccharides activates macrophages, we measured pinocytic activity of RAW 264.7 cells. Pinocytic activity of GP-activated macrophages was examined by the uptake of neutral red (0.075%). In the absence of any treatment, the pinocytic activity was low, whereas, a significant enhancement of pinocytic activity was observed in macrophages treated with both fractions (Table 3). The results show that a dose-dependent enhancement of pinocytic activity was described in macrophages treated with 5–50 μg/ml doses of GP-1, with the peak at the concentration of 50 μg/L; while with treatment concentration of GP-2 increasing, negative effect on the rate of pinocytic activity was noticed. Thus, the individual polysaccharide fractions showed a similar pattern with respect to their ability to induce cell proliferation by RAW 264.7 macrophages.

Besides the macrophage proliferation effects of mushroom polysaccharides, effects on macrophage-activating has also been reported. Indeed, a variety of plant polysaccharides have been reported to exhibit beneficial pharmacological effects via their ability to modulate macrophage function (Schepetkin & Quinn, 2006). One of the most distinguished features of macrophage activation would be an increase in pinocytic activity. Cheng et al. (2008) reported *Glycyrrhiza uralensis* fish polysaccharide could activate peritoneal macrophage and enhanced pincytic activity. Likewise, we also found macrophages were activated by *G. lucidum* polysaccharide fractions inducing enhancement of pincytic activity.

Table 3
Effects of different concentrations of *Ganoderma lucidum* polysaccharides on pinocytic activity in macrophages RAW 264.7. Cells were incubated for 24 h with the indicated concentrations of polysaccharide fractions. Values are mean ± SD of four replicates. Control cells were incubated with medium alone.

Treatment	Concentration (μg/ml)	OD (490 nm)	Pinocytic rate (%)
Control	–	0.125 ± 0.017	
LPS	2	0.211 ± 0.017	68.80
GP-1	50	0.178 ± 0.024**,a	42.67
	25	0.171 ± 0.022*,a	36.60
	5	0.172 ± 0.012**,a	37.80
GP-2	50	0.159 ± 0.016[a]	19.80
	25	0.187 ± 0.011**,a	49.33
	5	0.194 ± 0.009**	55.20

* $p < 0.05$.
** $p < 0.01$, significantly different from the control.
[a] $p < 0.01$, significantly different from LPS -treated cells as positive control.

3.5. In vitro growth inhibition of MDA

As recently demonstrated, some extracts of *G. lucidum* markedly inhibited growth of cancer cells directly, whereas other extracts showed another functions. For example, triterpenes in *G. lucidum* directly inhibit growth and invasive behavior of cancer cells (Lin, Li, Lee, & Kan, 2003), whereas *G. lucidum* immunomodulatory proteins stimulate immune system resulting in activation of murine splenocytes and presents no cytotoxicity in vitro (Sheu, Chien, Chien, Chen, & Chin, 2004). To determine which function the extracted polysaccharide of *G. lucidum* had, we researched the antitumor activity of *G. lucidum* polysaccharides and the co-cultured medium, named as GP1-MΦ-CM, GP2-MΦ-CM and N-MΦ-CM (control), according to the method of MTT.

Table 4 showed the inhibition activity of the polysaccharide fractions extracted and purified from the fruiting bodies of *G. luci-*

Table 4
Effects of different concentrations of *Ganoderma lucidum* polysaccharides and the co-cultured medium *Ganoderma lucidum* polysaccharides with macrophages on growth inhibition activity in MDA. Cells were incubated for 24 h with the indicated concentrations of polysaccharide fractions, and the indicated co-cultured medium, named as GP1-MΦ-CM, GP2-MΦ-CM and LPS-MΦ-CM. Values are mean ± SD of four replicates. Control cells were incubated with medium alone.

Treatment	Concentration (μg/ml)	OD (490 nm)	Inhibition ratio (%)
Control	–	0.353 ± 0.060	
GP-1	50	0.336 ± 0.053	4.81
	25	0.351 ± 0.056	0.71
	5	0.352 ± 0.016	0.50
GP-2	50	0.339 ± 0.015	4.13
	25	0.330 ± 0.014	6.72
	5	0.347 ± 0.009	1.86
N-MΦ-CM	–	0.264 ± 0.029	
LPS-MΦ-CM	2	0.102 ± 0.015	61.36
GP1-MΦ-CM	50	0.163 ± 0.013**,a	38.27
	25	0.168 ± 0.013**,a	36.12
	5	0.216 ± 0.022*,a	18.22
GP2-MΦ-CM	50	0.211 ± 0.019**,a	20.05
	25	0.153 ± 0.020**,a	42.03
	5	0.212 ± 0.019**,a	19.54

* $p < 0.05$.
** $p < 0.01$, significantly different from N-MΦ-CM.
[a] $p < 0.01$, significantly different from LPS -treated cells as positive control.

dum. The fractions GP-1 and GP-2 at the concentrations (5–50 μg/ml) showed very poor inhibition effect on MDA cells. The extract GP-2 at a concentration of 25 μg/ml, inhibited cancer cells by 6.72%, while GP-1 exhibited a lower inhibition rate by 4.81% at the concentration of 50 μg/L. The results indicated that the antitumor activity of the polysaccharides fractions on the breast cancer cells was not directly killing way but other mechanism involved.

For further explanation of the inhibitory effect of polysaccharides fractions on the breast cancer cells, we also analyzed the effects of co-medium which was treated by polysaccharides and macrophages. As shown in Table 4, both GP1-MΦ-CM and GP2-MΦ-CM resulted in a significant growth inhibition of cancer cells MDA. As same as the effect of *G. lucidum* polysaccharides on proliferation and pinocytic activity in macrophages, the GP2-MΦ-CM showed a better effect on cancer cells inhibition. The highest inhibition rate of MDA cells by GP2-MΦ-CM was 42.03% of that induced by 25 μg/ml polysaccharide GP-2, whereas the rate by GP1-MΦ-CM was 38.27%, which induced by 50 μg/ml polysaccharide GP-2. To sum up, *G. lucidum* polysaccharides and macrophages co-culture medium had the significant growth inhibition on breast cancer cells compared to control cells and the polysaccharides treated cells.

Macrophages play critical roles in host defense, including phagocytosis of pathogens and apoptotic cell, production of cytokines, and proteolytic processing and presentation of foreign antigens. Our findings indicated that both fractions possessed a proliferative effect on macrophage up to 160% of the control cells. These results provoked a question whether this enhanced proliferation was concomitantly associated with induced macrophage functions in case of inflammation. To answer this question we examined some of these functions. Interestingly, the treatment with GP-1 and GP-2 resulted in a dramatic pinocytic activity ($P < 0.01$) as high as 42% and 55%, respectively (Table 3).

Moreover, protective action of the polysaccharide is not limited to the enhancement of the macrophage phagocytic activity alone. Stimulated macrophages also release a broad spectrum of cytokines including interleukins, TNF-α (Zheng, Su, Dai, & Wu, 2005) and NO (Gamal-Eldeen, Ahmed, & Abo-Zeid, 2009; Lee et al., 2009), which are the inhibitory factors of cancer. In the present research, polysaccharides (GP-1 and GP-2) and macrophages co-culture medium dramatically ($P < 0.01$) inhibited cancer cell proliferation to 38% and 42% of non-polysaccharide treated medium, respectively, while two fractions signed absolutely no directly inhibitory activity on cancer cells (Table 4). The results indicated that the antitumor factors induced from macrophage by polysaccharides fractions were required for GP-1 and GP-2 antitumor activity.

In the results of the in vitro immunity and antitumor activities, both polysaccharides having MW 1.926 and 1086 kDa, respectively, exhibited potent in vitro immune activation, and antitumor activity on the breast cancer cells. Compared with polysaccharides GP-1, GP-2 having a high MW showed a stronger activity. The high molecular weight polysaccharides is considered to be a consequence of stimulation of the immune response in the host, rather than direct killing tumor cells, as reported by JK (2004) and Wasser (2002).

4. Conclusion

Previous studies indicated that some of the biological properties of *G. lucidum* were due to the presence of low-molecular weight compounds, such as protein and triterpenoid, we have demonstrated here that polysaccharides of *G. lucidum* have potent immunomodulatory properties. According to our experiment, two *G. lucidum* polysaccharide fractions, with the MW 1.926 and 1086 kDa, were obtained. And we demonstrated that these polysaccharides exhibited immunomodulatory activity, including promoted proliferative effect and pinocytic responses of macrophage. These activities could lead to enhanced host defence and inhibited the growth of cancer cell. Further studies are needed to investigate the physiological and pharmacological properties of the *G. lucidum* polysaccharides, which is of potential research and development value in the field of pharmaceutical and functional foods.

Acknowledgment

This work was supported by the earmarked fund for Modern Agro-industry Technology Research System, National Key Technology R&D Program (2008BADA1B06) and a grant-in-aid for Youth Innovation Fund from Nanjing Agricultural University, China (No. Y200720).

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