Bioresource Technology 100 (2009) 1860–1863

Contents lists available at ScienceDirect

Bioresource Technology

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

Short Communication

Structural elucidation and immunological activity of a polysaccharide from the fruiting body of *Armillaria mellea*

Yongxu Sun [a,*], Haitao Liang [b], Xiantao Zhang [c], Haibin Tong [d], Jicheng Liu [a,*]

[a] Department of Pharmacology, Qiqihar Medical University, Qiqihar 161042, China
[b] Transplantation Biology Research Division, State Key Laboratory of Biomembrane and Membrane Biotechnology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China
[c] New Medicine Development Limited Company, Jilin Revision Pharmaceutical Industry, Changchun 130012, China
[d] Life Science Research Center, Beihua University, Jilin 132013, China

ARTICLE INFO

Article history:
Received 19 August 2008
Received in revised form 18 September 2008
Accepted 19 September 2008
Available online 17 November 2008

Keywords:
Armillaria mellea
Polysaccharide
Structural analysis
NMR spectroscopy
Lymphocyte proliferation

ABSTRACT

The water-soluble polysaccharide (AMP), with a molecular mass of 7.8×10^3 Da as determined by high-performance size-exclusion chromatography (HPSEC), was obtained from the fruiting body of *Armillaria mellea*. Methylation, Smith degradation, acetolysis, ^{1}H and ^{13}C NMR spectroscopy and acid hydrolysis studies were conducted to elucidate its structure. The results indicated that AMP consisted of a backbone composed of (1→6)-linked-α-D-glucopyranosyl, (1→2,6)-linked-α-D-glucopyranosyl and (1→6)-linked-α-D-galactopyranosyl residues in the ratio of 3:1:1, and terminated with one single terminal (1→)-β-D-glucopyranosyl at the O-2 position of (1→2,6)-linked-α-D-glucopyranosyl, on average, along the main chain. Preliminary tests in vitro showed that AMP has stimulating effects on murine lymphocyte proliferation induced by concanavalin A or lipopolysaccharide in a dose-dependent manner. It is a possible potential immunopotentiating agent for use in health-care food or medicine.

© 2008 Elsevier Ltd. All rights reserved.

1. Introduction

Armillaria mellea is a traditional Chinese medicinal and edible fungus distributed in the Northeast Provinces of China, which is a Tricholomataceae fungus belonging to the Basidiomycotina. *A. mellea* accretes with the famous Chinese traditional medicine Rhizoma Gastrodiae. The substance distilled from *A. mellea* has the similar pharmacological activity and clinical curative effect to that of Rhizoma Gastrodiae, and has no poisonous effect or side effect. The substance has the functions of anti-tumor, anti-inflammation, anti-radiation and immunomodulation (Yu et al., 2001; Lin et al., 1988). So far there is not any information published about the polysaccharide isolated from the fruiting body of this fungus. Therefore, this paper was concerned with the isolation, structural elucidation of a water-soluble polysaccharide from the fruiting body of *A. mellea* and with the evaluation of the possible immunomodulatory activity in vitro by ConA or Lps-induced lymphocyte proliferation test.

2. Methods

2.1. Chemicals

Sepharose CL-6B, DEAE-Sephadex A-25 and Sephadex G-25 were purchased from Pharmacia Biotech. Trifluoroacetic acid (TFA) and Me_2SO were purchased from E. Merck. Ovalbumin (OVA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Concanavalin A (ConA) and lipopolysaccharide (LPS) were from Sigma Chemical Co. Fetal calf serum (FCS) was provided by Hangzhou Sijiqing Co. Medium RPMI-1640 was purchased from Gibco Invitrogen Co. The RMPI-1640 medium, used for immunological tests, was supplemented with HEPES buffer 10 μmol/ml, penicillin 100 IU/ml, streptomycin 100 μg/ml, l-glutamine 2 μmol/ml, 2-mercaptoethanol 50 μmol/l and 10% newborn bovine serum, pH 7.2. All other reagents were of grade AR.

2.2. General methods

The specific rotation was determined at 20 ± 1 °C with a WZZ-T1 Polarimeter (Shanghai Physical Optics Instrument Co.). UV–Vis absorption spectra were recorded with a Shimadzu MPS-2000 spectrophotometer. GC was performed on a Vavian 3400 instrument (Hewlett–Packard Component, USA) equipped with SE-30 column (50 mm × 0.20 mm × 0.25μm). The column temperature

* Corresponding authors. Tel.: +86 452 6731303; fax: +86 452 6727648 (Y. Sun).
E-mail addresses: yongxusun1978@yahoo.com.cn (Y. Sun), jichengliu@yahoo.cn (J. Liu).

0960-8524/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2008.09.036

was maintained at 120 °C for 2 min, and then increased to 250 °C for 3 min at a rate of 8 °C/min. Gas chromatography–mass spectrometry (GC–MS) was done on a HP5890 (II) instrument (Hewlett–Packard Component, USA) with an HPS quartz capillary column (25 m × 0.22 mm × 0.2 nm), and at temperatures programmed from 120 to 140 °C at 1 °C/min. The FTIR spectra (KBr pellets) were recorded on SPECORD in a range of 400–4000 cm^{-1}. Total carbohydrate content was determined by the Dubois's method (Dubois et al., 1956), using D-glucose as the standard. Protein was measured by the Lowry method (Lowry et al., 1951), using bovine serum albumin as a standard. Dialysis was carried out by using dialysis tubing (Spectra/Por MWCO: 500).

2.3. Extraction and purification of polysaccharide

The fruiting body of *A. mellea* was extracted with 3 volume of 95% EtOH at 75 °C for 5 h under reflux to remove lipid, and the supernatant was removed. The residue was then extracted with 10 volume of distilled water at 75 °C for 4 times and 3 h for each time. After centrifugation (1700*g* for 10 min, at 20 °C), the supernatant was concentrated 10-fold, and precipitated with 95% EtOH (1:5, v/v) at 4 °C for 12 h. The precipitate collected by centrifugation was suspended in distilled water to remove the protein by the Sevag method (Sun et al., 2008a,b), and exhaustively dialyzed against water for 2 days. Then the concentrated dialyzate was precipitated with 0.5, 1 and 3 volume of 95% EtOH one after another. The precipitate with 3 volume of 95% EtOH was washed with absolute ethanol, acetone and ether, respectively (Chi et al., 2007). The washed precipitate was the crude polysaccharide, named as CAMP.

The CAMP was purified on the auto liquid chromatographic fractionation apparatus (MF99-1) made in shanghai city of China. The CAMP (100 mg) was dissolved in distilled water, centrifuged, and then the supernatant was applied to a column of DEAE-Sephadex A-25 equilibrated with 0.9% NaCl. After loading with sample, the column was eluted with different concentrations of NaCl aqueous solution (0.15 and 3.9 M) stepwise at 8 ml/12 min. Test tubes (100 containing 8 ml eluant each) were collected using an automated step-by-step fraction collector. Total carbohydrate and protein content of each tube were measured by Dubois's and Lowry's method, respectively. The eluted solution was only separated into one fraction, and then purified by gel-permeation chromatography on a Sepharose CL-6B column (90 × 2cm), loading 100 mg the above-purified fraction for each run. The column was eluted with 0.9% NaCl with a flow rate of 0.5 ml/min. Fractions (test tube Nos. 38–41) containing a large amount of sugar were applied to a Sephadex G-25 column to remove salts, and freeze dried to obtain purified polysaccharide (78 mg), named as AMP.

2.4. Monosaccharide composition, properties and molecular weight determination

Gas chromatography (GC) was used for identification and quantification of the monosaccharides. AMP was hydrolyzed with 2 M TFA at 110 °C for 4 h (Parikh and Madamwar, 2006). The monosaccharides were conventionally converted into the alditol acetates as described (Oades, 1967; Johnes and Albersheim, 1972) and were analyzed by GC as foresaid. The absolute configurations of the monosaccharides were determined as described by Vliegenthart and co-workers using (+)-2-butanol (Gerwig et al., 1979).

The average molecular weight of AMP was determined by high-performance size-exclusion chromatography (HPSEC) (Cui et al., 2007), which was performed on a SHIMADZU HPLC system fitted with one TSK-G30007 column (7.8 mm ID × 30.0 cm l), a Lc-10ATvp pump, a SHIMADZU RID-10A detector and an on-line de-gasser. The data were processed by GPC processing software (Millennium[32] version). The mobile phase was 0.7% Na_2SO_4, and the flow rate was 0.5 ml min^{-1} at 40 °C, with 1.6 mpa. A sample (3 mg) was dissolved in the mobile phase (0.5 ml) and centrifuged (10,000 rpm, 3 min), and 20 μl of supernatant was injected in each run. The molecular mass was estimated by reference to a calibration curve made from a set of Dextran T-series standards of known molecular mass (T-700, 580, 470, 350, 280, 50, 25, 12, 10).

2.5. Partial hydrolysis with acid

The AMP (100 mg) was hydrolyzed with 0.05 M trifluoroacetic acid (2 ml), maintained at 95 °C for 16 h, centrifuged. After TFA was removed by evaporation, the remains were dialyzed with distilled water for 48 h, and then diluted the solution in the sack with ethanol. After hydrolyzation, the precipitate and supernatant in the sack and the fraction out of sack were dried and analyzed by GC as the alditol acetate. The precipitate, in the sack, was subjected to monosaccharide composition analysis of backbone and methylation analysis (Sun et al., 2008a,b).

2.6. Periodate oxidation–Smith degradation

For analytical purpose, 25 mg of the polysaccharide was dissolved in 12.5 ml of distilled water and 12.5 ml of 30 mmol/l $NaIO_4$ was added. The solution was maintained at 4 °C for 7 days in the dark, and 0.1 ml aliquots were withdrawn at 3–6 h intervals, diluted to 25 ml with distilled water and read in a spectrophometer at 223 nm (Linker et al., 2001). Excess periodate was decomposed by the addition of ethylene glycol (2 ml). The solution of periodate product (2 ml) was sampled to calculate the yield of formic acid by 0.01 M NaOH. The rest was dialyzed against distilled H_2O for 24 h. The solution was concentrated and reduced with $NaBH_4$ (60 mg), and the mixture was left for 24 h at room temperature, neutralized to pH 6.0 with 50% acetic acid, dialyzed as described above, and was concentrated to a volume (10 ml). One-third of solution described above was freeze dried and analyzed with GC. Others were added to the same volume of 1 M sulfuric acid, maintained for 40 h at 25 °C, neutralized to pH 6.0 with barium carbonate, and filtered. The filtrate was dialyzed as foresaid, and the content out of the sack was desiccated for GC analysis; the content inside was diluted with ethanol, and after centrifugation, the supernatant and precipitate were also dried out for the GC analysis.

2.7. Methylation analysis

The sample (20 mg) was methylated thrice according to Needs and Selvendran (1993). Complete methylation was confirmed by the disappearance of the OH band (3200–3700 cm^{-1}) in the IR spectrum. The methylated products were hydrolyzed, reduced and acetylated as described by Sweet et al. (1975). The partially methylated alditol acetates were analyzed by GC–MS under the same chromatographic conditions as above.

2.8. NMR spectroscopy

For NMR measurements, AMP was dried in a vacuum over P_2O_5 for several days, and then exchanged with deuterium (Dueñas-Chasco et al., 1997) by lyophilizing with D_2O for several times. The deuterium-exchanged polysaccharide (50 mg) was put in a 5-mm NMR tube and dissolved in 0.7 ml of 99.96% D_2O. Spectra were recorded with a Bruker AV-400 spectrometer. The ^1H and ^{13}C NMR spectra were recorded at 50 °C. Acetone was used as an internal standard (δ 31.09 ppm) for the ^{13}C spectrum. The ^1H NMR spectrum was recorded by fixing the HOD signal at δ 4.54 ppm at 50 °C.

2.9. Immunobiological activity assay

Male Kunming mice (GradeII, 8–12 weeks old) were purchased from the Pharmacology Experimental Center of Jilin University and were acclimatized for 1 week prior to use. All mice were housed under the standard conditions at 24 ± 1 °C, with humidity of 50 ± 10%, and a 12/12 h light/dark cycle. Rodent laboratory chow pellets and tap water were supplied ad libitum. All the procedures conducted by the Institute for Experimental Animals of Jilin University were carried out in strict accordance with the PR China legislation on the use and care of laboratory animals, and were approved by the university committee for animal experiments.

Spleen cells of mice were obtained by gently teasing the organ in RPMI-1640 medium. To isolate mononuclear cells, 5 ml aliquots of the spleen cell suspension were layered onto 2.5 ml aliquots of a polysucrose–sodium ditrizoate solution, and were centrifuged at 3000 rpm for 20 min at room temperature. Mononuclear cells were gently removed from the interface between medium and histopaque, and were transferred to a sterile container and washed with RPMI-1640. At last, the cells were resuspended in 5 ml RPMI-1640 medium, and cell counts were done. An aliquot of 100 μl of splenocytes mixed with the polysaccharide (50, 100, 200 μg/ml, final concentration) was seeded into each well of a 96-well plate in the presence of ConA (5.0 μg/ml) or LPS (10.0 μg/ml). After preincubation for three days at 37 °C in a humidified 5% CO_2 incubator, 10.0 μl of 0.4% MTT was added into each well (Sun and Liu, 2008). The plate was incubated for another 4 h, and then a total of 100 μl Me_2SO was added to the culture and homogenized for at least 10 min to fully dissolve the colored material. The absorbance at 570 nm was measured on an ELISA reader (Model 680, Bio-RAD Instruments). The control experiments were performed without the polysaccharide. Each experiment was performed in triplicate.

3. Results and discussion

3.1. Isolation, purification and structural analysis of polysaccharides

The AMP showed a single and symmetrically sharp peak, indicating its homogeneity on HPSEC (data not shown). According to the retention time, its molecular weight was estimated to be 7.8×10^3 Da, and it showed an $[\alpha]_D^{20}$ = +55.1 (*c* 0.295, H_2O). It had a negative response to the Bradford test and no absorption at 280 or 260 nm in the UV spectrum, indicating the absence of protein and nucleic acid. Total carbohydrate content was determined to be 94%. The AMP was composed of D-glucose and D-galactose as detected by GC in the ratio of 5:1. The absolute configuration of the monosaccharides were determined by the GC examination of acetylated (+)-2-octyl glycosides, and showed that all have D configurations.

The FTIR spectra of AMP are shown in Table 1. The bands in the region of 3409.01 cm^{-1} are due to the hydroxyl stretching vibration of the polysaccharides. The bands in the region of 2935.36 cm^{-1} are due to C–H stretching vibration, and the bands in the region of 1632.93 cm^{-1} are due to associated water. Moreover, the characteristic absorptions at 849.78 and 890.86 cm^{-1} in the IR spectra indicated that α- and β-configurations are simultaneously existing in AMP (Zhang, 1999).

The GC–MS results (Table 2) indicated that the backbone chains are mainly (1→6)-linked-α-D-glucopyranosyl (Residue-A), (1→2,6)-linked-α-D-glucopyranosyl (Residue-B) and (1→6)-linked-α-D-galactopyranosyl residues (Residue-C). The side chains attached to the O-2 position of Residue-B contained single terminal (1→)-β-D-glucopyranosyl (Residue-D) groups. According to the peak areas, four types of residues are in the ratio of 3:1:1:1. This was also in accordance with the results of the periodate oxidation and Smith degradation. Supporting the results of methylation analysis, GC of the products that was obtained from Periodate oxidation–Smith degradation only showed the presence of glycerol.

In the anomeric region of the ^{1}H NMR spectrum (Table 1) of AMP, four signals occurred at δ 5.01, δ 5.08, δ 5.02 and δ 4.73 ppm, which were assigned as Residue-A, Residue-B, Residue-C and Residue-D, respectively. And accordingly in the anomeric region of the ^{13}C NMR spectrum, four carbon resonances appeared at δ 99.20, δ 98.20, δ 100.50 and δ 106.05 ppm. All the results confirmed the presence of four sugar residues and their configurations: Residues-A, B and C are α-configurations, and Residue-D is form of β-configuration, consistent with GC and FTIR data. In the high magnetic field, the δ 78.31 signal should come from C-2 resonance of Residue-B. C-6 chemical shifts of Residue-A, Residue-B, Residue-C and Residue-D occurred at δ 67.31, δ 66.25, δ 69.12, δ 60.02, respectively. All the NMR chemical shifts were compared with the literature values (Zhao et al., 2006; Gao et al., 2006; Fan et al., 2006; Ye et al., 2008; Rout et al., 2006; Dueñas-Chasco et al., 1998).

The structure elucidation of an immunoregulatory polysaccharide from the fruiting body of *A. mellea,* by means of chemical analyzes and NMR spectroscopy, was studied for the first time. The structure of this polysaccharide can be demonstrated as follows: the backbone of AMP was composed of (1→6)-linked-α-D-glucopyranosyl, (1→2,6)-linked-α-D-glucopyranosyl and (1→6)-linked-α-D-galactopyranosyl residues in the ratio of 3:1:1, and terminated with one single terminal (1→)-β-D-glucopyranosyl at the O-2 position of (1→2,6)-linked-α-D-glucopyranosyl along the main chain.

3.2. Immunological activity of AMP

Lymphocyte proliferation is a crucial event in the activation cascade of both cellular and humoral immune responses. In order to investigate a possible immunomodulatory effect of the polysaccharides, contaminant endotoxins were removed from the polysaccharide preparations by affinity chromatography in a polymyxin B-coupled column. Spleen lymphocyte proliferation induced by ConA in vitro maybe used as a method to evaluate T lymphocyte activity, while that induced by LPS maybe used to examine B lymphocyte activity (Sun et al., 2008a,b). As observed in Table 3, CAMP and AMP could produce significant dose-dependent increases in ConA or LPS-induced spleen lymphocytes proliferation ($P < 0.05$ or $P < 0.01$). Therefore, we can draw a conclusion that CAMP or AMP is possible potential immunopotentiating agent for use in healthcare food or medicine.

Table 1
The data of UV analysis, IR analysis and NMR analysis of AMP

Assay	Peaks or signals at
UV analysis (nm)	210
IR analysis (cm^{-1})	3409.01; 2935.36; 1632.93; 890.86; 849.78
^{1}H NMR analysis (ppm)	5.08; 5.02; 5.01; 4.73
^{13}C NMR analysis (ppm)	106.05; 100.50; 99.20; 98.20; 78.31; 69.12; 67.31; 66.25; 60.02

Table 2
The results of methylation analysis of AMP

Peak no.	Methylated sugar	Molar ratio	Linkage type
1 (Residue-A)	2,3,4,-Me_3-Glcp	3	→6)-α-Glcp-(1→
2 (Residue-B)	3,4-Me_2-Glcp	1	→2,6)-α-Glcp-(1→
3 (Residue-C)	2,3,4,-Me_3-Gap*l*	1	→6)-α-Gal*p*-(1→
4 (Residue-D)	2,3,4,6-Me_4-Glc*p*	1	β-Glc*p*-(1→

Table 3
Effects of the polysaccharides from the fruiting body of *Armillaria mellea* on ConA- or LPS-induced proliferation activity of mouse splenocytes in vitro[a]

Preparation	Concentrations (μg/ml)	ConA (SI)	LPS (SI)
Control	–	1.00 ± 0.05	1.00 ± 0.03
CAMP	50	1.22 ± 0.04[b]	1.23 ± 0.03[b]
	100	1.26 ± 0.02[b]	1.37 ± 0.05[c]
	200	1.39 ± 0.03[c]	1.40 ± 0.01[c]
AMP	50	1.25 ± 0.06[b]	1.36 ± 0.03[c]
	100	1.48 ± 0.05[c]	1.46 ± 0.04[c]
	200	1.63 ± 0.04[c]	1.62 ± 0.07[c]

Crude polysaccharide was named as CAMP.
Purified polysaccharide was named as AMP.
[a] Splenocyte proliferation was measured by the MTT method as described in the text, and shown as a stimulation index (SI). The results are represented as mean ± S.D. based on the three independent experiments.
[b] $P < 0.05$, significantly different from the control.
[c] $P < 0.01$, significantly different from the control.

Acknowledgements

This study was supported by the Research Fund of the Qiqihar Medical University. The authors are deeply grateful to Prof. Da-you Liu at Changchun University of Traditional Chinese Medicine for identification of *A. mellea.*

References

Chi, Z., Su, C.D., Lu, W.D., 2007. A new exopolysaccharide produced by marine *Cyanothece* sp. 113. Bioresour. Technol. 98, 1329–1332.
Cui, F.J., Tao, W.Y., Xu, Z.H., Guo, W.J., Xu, H.Y., Ao, Z.H., Jin, J., Wei, Y.Q., 2007. Structural analysis of anti-tumor heteropolysaccharide GFPS1b from the cultured mycelia of *Grifola frondosa* GF9801. Bioresour. Technol. 98, 395–401.
Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356.
Dueñas-Chasco, M., Rodrı́guez-Carvajal, M.A., Tejero-Mateo, P., Espartero, J.L., Irastorza-Iribas, A., Gil-Serrano, A.M., 1998. Structural analysis of the exopolysaccharides produced by *Lactobacillus* spp. G-77. Carbohydr. Res. 307, 125–133.
Dueñas-Chasco, M.T., Rodríguez-Carvajal, M.A., Mateo, P.T., Franco-Rodríguez, G., Espartero, J., Irastorza-Iribas, A., Gil-Serrano, A.M., 1997. Structural analysis of the exopolysaccharide produced by *Pediococcus damnosus*. Carbohydr. Res. 303, 453–458.
Fan, J.M., Zhang, J.S., Tang, Q.J., Liu, Y.F., Zhang, A.Q., Pan, Y.J., 2006. Structural elucidation of a neutral fucogalactan from the mycelium of *Coprinus comatus*. Carbohydr. Res. 341, 1130–1134.
Gao, W., Li, X.Q., Liu, L., Wang, M.C., Fan, H.T., Li, C., Lv, Z.G., Wang, X.J., Mei, Q.B., 2006. Structural analysis of water-soluble glucans from the root of *Angelica sinensis* (Oliv.) diels. Carbohydr. Res. 341, 1870–1877.
Gerwig, G.J., Kamerling, J.P., Vliegenthart, J.F.G., 1979. Determination of the absolute configuration of monosaccharides in complex carbohydrates by capillary g.l.c.. Carbohydr. Res. 77, 1–7.
Johnes, T.M., Albersheim, P., 1972. A gas chromatographic method for the determination of aldose and uronic acid constituents of plant cell wall polysaccharide. Plant Physiol. 49, 926–936.
Linker, A., Evans, L.R., Impallomeni, G., 2001. The structure of a polysaccharide from infectious strains of *Burkholderia cepacia*. Carbohydr. Res. 335, 45–54.
Lin, Z.B., Zhang, D.M., Xia, D., 1988. Effect of *Armillaria mellea* polysaccharide on immunity in mice. Chin. Pharm. Bul. 4, 93–96.
Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.
Needs, P.W., Selvendran, R.R., 1993. Avoiding oxidative degradation during sodium hydroxide/methyl iodide-mediated carbohydrate methylation in dimethyl sulfoxide. Carbohydr. Res. 245, 1–10.
Oades, J.M., 1967. Gas-liquid chromatography of alditol acetates and its application to the analysis of sugars in complex hydrolysates. J. Chromatogr. 28, 246–252.
Parikh, A., Madamwar, D., 2006. Partial characterization of extracellular polysaccharides from cyanobacteria. Bioresour. Technol. 97, 1822–1827.
Rout, D., Mondal, S., Chakraborty, I., Islam, S.S., 2006. The structure of a polysaccharide from Fraction-II of an edible mushroom, *Pleurotus florida*. Carbohydr. Res. 341, 995–1002.
Sun, Y.X., Li, M.Q., Liu, J.C., 2008a. Haemolytic activities and adjuvant effect of *Anemone raddeana* saponins (ARS) on the immune responses to ovalbumin in mice. Int. Immunopharmacol. 8, 1095–1102.
Sun, Y.X., Wang, S.S., Li, T.B., Li, X., Jiao, L.L., Zhang, L.P., 2008b. Purification, structure and immunobiological activity of a new water-soluble polysaccharide from the mycelium of *Polyporus albicans* (Imaz) Teng. Bioresour. Technol. 99, 900–904.
Sun, Y.X., Liu, J.C., 2008. Adjuvant effect of water-soluble polysaccharide (PAP) from the mycelium of *Polyporus albicans* on the immune responses to ovalbumin in mice. Vaccine 26, 3932–3936.
Sweet, D.P., Shapiro, R.H., Albersheim, P., 1975. Quantitative analysis by various g.l.c. response-factor theories for partially methylated and partially ethylated alditol acetates. Carbohydr. Res. 40, 217–225.
Ye, L.B., Zhang, J.S., Ye, X.J., Tang, Q.J., Liu, Y.F., Gong, C.Y., Du, X.J., Pan, Y.J., 2008. Structural elucidation of the polysaccharide moiety of a glycopeptide (GLPCW-II) from Ganoderma lucidum fruiting bodies. Carbohydr. Res. 343, 746–752.
Yu, M., Sheng, Y.S., Mei, Y.D., 2001. Immunological enhancement research on the polysaccharide of *Armillaria mellea*. Chin. J. Biol. 18, 16–18.
Zhang, Wei-Jie, 1999. Biochemical Study Technology in Polysaccharide Compounds. Zhejiang University Press. pp. 193–197.
Zhao, C., Li, M., Luo, Y.F., Wu, W.K., 2006. Isolation and structural characterization of an immunostimulating polysaccharide form fuzi, *Aconitum carmichaeli*. Carbohydr. Res. 341, 485–491.