PERGAMON

Phytochemistry 59 (2002) 175–181

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Structural features of immunologically active polysaccharides from *Ganoderma lucidum*

Xing-Feng Bao, Xue-Song Wang, Qun Dong, Ji-Nian Fang*, Xiao-Yu Li

Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 294 Tai-yuan Road, Shanghai 200031, People's Republic of China

Received in revised form 6 September 2001

Abstract

Three polysaccharides, two heteroglycans (PL-1 and PL-4) and one glucan (PL-3), were solubilized from the fruit bodies of *Ganoderma lucidum* and isolated by anion-exchange and gel-filtration chromatography. Their structural features were elucidated by glycosyl residue and glycosyl linkage composition analyses, partial acid hydrolysis, acetolysis, periodate oxidation, 1D and 2D NMR spectroscopy, and ESI–MS experiments. The data obtained indicated that PL-1 had a backbone consisting of 1,4-linked α-D-glucopyranosyl residues and 1,6-linked β-D-galactopyranosyl residues with branches at *O*-6 of glucose residues and *O*-2 of galactose residues, composed of terminal glucose, 1,6-linked glucosyl residues and terminal rhamnose. PL-3 was a highly branched glucan composed of 1,3-linked β-D-glucopyranosyl residues substituted at *O*-6 with 1,6-linked glucosyl residues. PL-4 was comprised of 1,3-, 1,4-, 1,6-linked β-D-glucopyranosyl residues and 1,6-linked β-D-mannopyranosyl residues. These polysaccharides enhanced the proliferation of T- and B-lymphocytes in vitro to varying contents and PL-1 exhibited an immune-stimulating activity in mice.

Keywords: *Ganoderma lucidum*; Polyparaceae; Structural elucidation; Immunological activity; Heteroglycan; Glucan

1. Introduction

Ganoderma lucidum, an oriental fungus, has been widely used to promote health and longevity in China and other East Asian countries. The fruit bodies, cultured mycelia, and spores of *G. lucidum* were reported to be effective in the treatment of chronic hepatopathy, hypertension, hyperglycemia and neophasia (Franz, 1989; Furusawa et al., 1992; Shiao et al., 1994). This fungus has attracted considerable attention because its polysaccharides have anti-tumor (Miyazaki and Nishijima, 1981; Wang et al., 1997) and hypoglycemic (Hikino et al., 1985; Tomoda et al., 1986) activities. However, the wild mushroom of *G. lucidum* is scarce. Thus, we have an interest in the cultivated fungus and try to find immunological polysaccharides from it. Recently, we have reported an immunomodulating polysaccharide from the spores of *G. lucidum* (Bao et al., 2001). Now, we describe the isolation and structural elucidation of three neutral polysaccharides that have an immunoactivating activity from the fruit bodies of *G. lucidum*.

2. Results and discussion

A brownish crude polysaccharide fraction, named PL (yield: 3.8% from the crude material), was obtained from the dried fruit bodies of *G. lucidum* by hot water extraction. The crude product was fractionated on a DEAE–cellulose column with water and different concentrations of stepwise NaCl solution elution (0.05, 0.2 and 0.5 M NaCl), leading to the isolation of four polysaccharide sub fractions PL-1, PL-2, PL-3 and PL-4 (Fig. 1). HPSEC analysis showed that PL-2 consisted of more than 4 peaks with similar elution times and PL-1, PL-3 and PL-4 gave a dominant peak in contrast to other minor components. Therefore, each fraction besides PL-2 was further purified on a Sephacryl S-300 HR (or S-200 HR) column and gave a homogeneous polysaccharide.

PL-1 was composed of Rha, Gal and Glc in the molar ratios of ca. 1:4:13 (Table 1). Glc was the only monosaccharide detected in PL-3, while PL-4 contained Man

* Corresponding author. Tel.: +86-21-64311833x601; fax: +86-21-64370269.

E-mail address: jnfang@mail.shcnc.ac.cn (J.-N. Fang).

0031-9422/01/$ - see front matter
PII: S0031-9422(01)00450-2

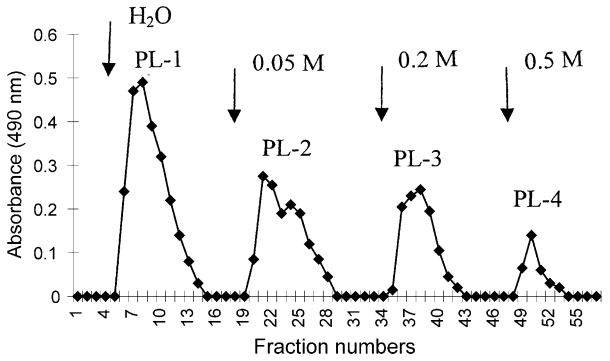

Fig. 1. DEAE–cellulose elution profile of the polysaccharides present in the aqueous extract of *Ganoderma lucidum* fruit bodies. The column was eluted stepwise with H_2O, 0.05, 0.1, 0.2 and 0.5 M NaCl solutions.

Table 1
Glycosyl residue compositions of PL-1, PL-1-A[a], PL-3 and PL-4 glycans

Residues	L-Rha	D-Man	D-Gal	D-Glc
PL-1	6	–	21	73
PL-1-A	–	–	24	76
PL-3	–	–	–	100
PL-4	–	7	–	93

[a] PL-1-A is the polymer obtained by partial acid hydrolysis of PL-1.

and Glc in a molar ratio of ca. 1:13 (Table 1). All glycosyl residues in PL-1, PL-3 and PL-4 were D except rhamnose that was L. PL-1, PL-3 and PL-4 had specific rotations $[\alpha]_D^{15}$ +129.6° (*c* 0.72, H_2O), $[\alpha]_D^{15}$ −52.7° (*c* 0.30, H_2O), $[\alpha]_D^{15}$ −38.3° (*c* 0.29, H_2O), respectively. The high positive value of the specific rotation of PL-1 and the characteristic absorption at ca. 845 cm^{-1} in the IR spectrum of PL-1 were indicative of the α glycosidic linkages of the glucosyl residues (Tsumuraya and Misaki, 1979), while the characteristic peak at ca. 890 cm^{-1} in the IR spectra of PL-3 and PL-4 indicated the glucosyl residues in PL-3 and PL-4 were β-linked (Ghosh and Rao, 1981). No absorption at 280 nm indicated all three polysaccharides did not contain any protein. The average M_rs of PL-1, PL-3 and PL-4 were estimated to be 8.3×10^3, 6.3×10^4 and 2.0×10^5, respectively, based on the calibration curve of the elution times of standard Dextrans on HPSEC.

The results of glycosyl linkage composition analyses (Table 2) indicated that PL-1, PL-3 and PL-4 were branched polysaccharides. The high content of 1,4-linked glucopyranosyl residues in PL-1 suggested that the backbone chain of PL-1 contained the 1,4-linked glucosyl residues with branches at *O*-6 of some glucosyl residues. Except for the presence of a small amount of 1,6-linked mannosyl residues in PL-4, both PL-3 and PL-4 had a complex glucan structure.

The ^1H and ^{13}C NMR spectral signals of PL-1, PL-3, and PL-4 were assigned using 2D NMR and referring to the previous reports (Bao and Fang, 2001; Paramonov et al., 1998; Bubb et al., 1997; Karlsson et al., 1997), and the anomeric signals and other characteristic signals were summarized in Table 3. The assignment of PL-1 was proved by the NMR spectrum of the partial hydrolyzed product PL-1-A, in which the signals from rhamnosyl residues and *O*-2 substituted galactosyl residues disappeared, while the signal for 1,6-linked galactosyl residues became stronger. As shown in Table 3, the anomeric configuration determination by NMR spectroscopic analysis was in accordance with the deduction from the [α] values and IR spectra. Also, the presence of *O*-substituted carbon signals confirmed the results of methylation analysis described above.

The hydrolysis of PL-1 with 0.05 M TFA at 100 C for 1 h gave a degraded polymer (PL-1-A) and a monosaccharide fraction. PL-1-A was shown to be a homogeneous polymer (M_r 6.1×10^3) by HPSEC. Glycosyl linkage analysis of PL-1-A (Table 2) revealed that the content of 1,6-linked galactosyl residues increased in comparison with that of PL-1, and that the terminal rhamnose

Table 2
Glycosyl linkage compositions of PL-1, PL-1-A [a], PL-1-B [b], PL-3 and PL-4 glycans

Components	Molar percent					Linkages
	PL-1	PL-3	PL-1-A	PL-1-B	PL-4	
2,3,4-Me_3-Rha	5	–	–	–	–	Rha_p-(1→
2,3,4,6-Me_4-Glc	14	15	19	21	17	Glc_p-(1→
2,3,6-Me_3-Glc	43	43	39	–	37	→4)-Glc_p-(1→
2,4,6-Me_3-Glc	–	–	–	20	11	→3)-Glc_p-(1→
2,3,4-Me_3-Glc	4	4	10	40	12	→6)-Glc_p-(1→
2,3,4-Me_3-Gal	16	24	12	–	–	→6)-Gal_p-(1→
2,3,4-Me_3-Man	–	–	–	–	7	→6)-Man_p-(1→
2,4-Me_2-Glc	–	–	–	19	4	→3,6)-Glc_p-(1→
2,3-Me_2-Glc	13	14	19	–	11	→4,6)-Glc_p-(1→
3,4-Me_2-Gal	5	–	–	–	–	→2,6)-Gal_p-(1→

[a] PL-1-A is the polymer generated by partial acid hydrolysis of PL-1.
[b] PL-1-B is the polymer generated by partial acid hydrolysis of PL-1-A.

Table 3
Characteristic signals in the ^{1}H and ^{13}C NMR spectra of PL-1, PL-3 and PL-4[a]

Polysaccharides	Chemical shifts (H/C) (ppm)	Hydrogen/carbon assignments
PL-1	5.48/101.9	H1/C-1 of 1,4-linked or terminal α-D-Glc$_p$
	4.60/104.7	H1/C-1 of 1,6-linked β-D-Gal$_p$
	4.60/103.6	H1/C-1 of 1,2,6-linked β-D-Gal$_p$
	5.05/100.0	H1/C-1 of 1,6- or 1,4,6-linked α-D-Glc$_p$
	5.14/97.9	H1/C-1 of α-L-Rha$_p$
	80.1	C-2 of *O*-2 substituted β-D-Gal
	79.0	C-4 of *O*-4 substituted α-D-Glc
	68.9	C-6 of *O*-6 substituted β-D-Gal
	68.7	C-6 of *O*-6 substituted α-D-Glc
	1.32/17.9	H6/C-6 of α-L-Rha
PL-3	4.62/104.8	H1/C-1 of β-D-Glc$_p$
	86.3	C-3 of *O*-3 substituted β-D-Glc
	69.2	C-6 of *O*-6 substituted β-D-Glc
PL-4	4.62/104.8	H1/C-1 of β-D-Glc$_p$
	n.r.[b]/101.7	H1/C-1 of β-D-Man$_p$
	86.3	C-3 of *O*-3 substituted β-D-Glc
	80.4	C-4 of *O*-4 substituted β-D-Glc
	69.2	C-6 of *O*-6 substituted β-D-Glc
	68.4	C-6 of *O*-6 substituted β-D-Man

[a] Chemical shifts in ppm relative to CH_3OH at δ 51.07 for ^{13}C at room temperature in D_2O.
[b] n.r., Not resolved.

and 1,2,6-linked galactosyl residues disappeared. The monosaccharide fraction mainly contained rhamnose and a small amount of glucose and galactose. These results indicated that the terminal rhamnose was linked to *O*-2 of 1,6-linked galactosyl residues. PL-1-A was further hydrolyzed with 0.1 M TFA at 100 °C for 1.5 h, giving the degraded polymer (PL-1-B) and three oligosaccharide fractions (PO1, PO2 and PO3). Glycosyl linkage analysis of PL-1-B (Table 2) revealed that the content of 1,6-linked galactosyl residues decreased compared with that of PL-1-A, indicating that the backbone of PL-1 was partly fragmented. The smallest M_r fraction (PO1) eluted from a Sephadex G-10 column was mixed monosaccharides and not studied further. Glycosyl residue composition analyses of the PO2 and PO3 fractions indicated that both were comprised of glucose. The ESI–MS spectra of PO2 and PO3 fractions revealed that PO2 contained a disaccharide and PO3 contained a tetrasaccharide, both composed of hexosyl residues. 2D NMR spectroscopic experiments established that oligosaccharides PO2 and PO3 had the following structures, respectively: α-D-Glc$_p$-(1→4)-α-D-Glc$_p$ (PO2) and α-D-Glc$_p$-(1→6)-α-D-Glc$_p$-(1→4)-α-D-Glc$_p$- (1→4)-α-D-Glc$_p$ (PO3).

Three oligosaccharide fractions (AO1, AO2 and AO3) were obtained by Sephadex G-10 chromatography after acetolysis of PL-1. The first fraction (AO1) contained a mixture of glucose, galactose and rhamnose. AO2 and AO3 were identified as a disaccharide and trisaccharide, respectively, composed of 1,4-linked Glc$_p$ by the 2D NMR spectroscopy and ESI–MS experiments. These results suggested that PL-1 had a backbone mainly composed of 1,4-linked glucosyl residues, and that 1,6-linked glycosyl residues were dispersed in the backbone chain. With the consideration of the low content of 1,6-linked glucosyl residues in PL-1, 1,6-linked galactosyl residues occurred in the main chain of PL-1.

PL-3 and PL-4 were oxidized with periodate, and the products were reduced, hydrolyzed and analyzed. The unoxidized sugar was glucose in both samples, and the yields were 21.5% in PL-3 and 17.6% in PL-4. PL-3 consumed 1.22 mol of periodate, with commitant production of 0.56 mol of formic acid per sugar residue. Also PL-4 consumed 0.88 mol periodate and produced 0.27 mol formic acid for each hexosyl residue.

Taken together, our results suggested that PL-1 had a backbone composed of 1,4-linked α-D-glucopyranosyl residues and 1,6-linked β-D-galactopyranosyl residues with branches comprised of terminal glucose, 1,6-linked α-D-glucopranosyl residues linked to *O*-6 of 1,4-linked glucosyl residues, and terminal rhamnose linked to *O*-2 of 1,6-linked galactosyl residues. PL-3 glucan possessed a backbone composed of 1,3-linked β-D-glucopyranosyl residues with branching points at *O*-6 of glucosyl residues with 1,6-linked β-D-glucose side chains. Heteroglucan PL-4 consisted of a primary structure of 1,3-, and 1,4-linked β-D-glucopyranosyl residues and 1,6-linked β-D-mannopyranosyl residues with branches at *O*-6 of glucosyl residues composed of 1,6-linked β-D-glucopyranosyl residues.

Based on the earlier reports of anti-tumor and immunomodulating activities of *G. lucidum*, the immunological properties of PL-1, PL-3 and PL-4 were investigated. These polysaccharides all enhanced the proliferation of T- and B-lymphocytes to some extent in vitro (Fig. 2),

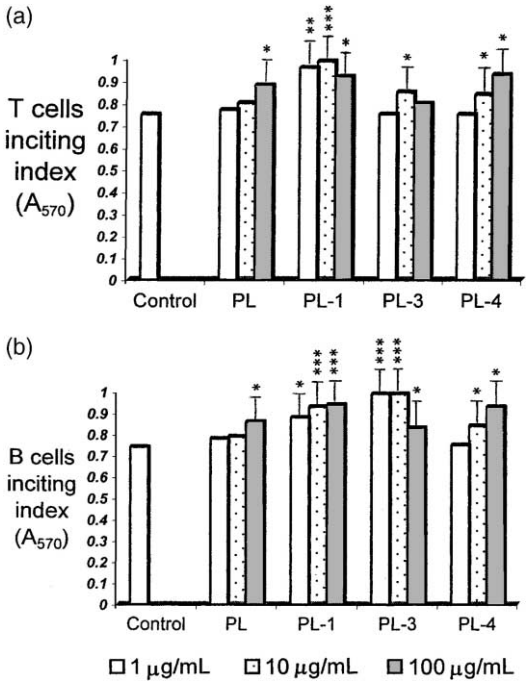

Fig. 2. (a) Effect of PL, PL-1, PL-3 and PL-4 on proliferation of ConA induced T lymphocyte in vitro. (b) Effect of PL, PL-1, PL-3 and PL-4 on proliferation of LPS induced B lymphocyte in vitro. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, significant difference from the control group.

and it was evident that PL-1 was more effective that PL-3 and PL-4. Therefore, the immunostimulating activity of PL-1 was further investigated in mice (Table 4). The data showed that PL-1 stimulated the proliferation of T- and B-lymphocytes and the production of antibodies, but had little effect on serum IgG, and complement (C 3) levels at a dose of 25 mg/kg.

In conclusion, the structural features of PL-1 were different from those of the polysaccharides that had been isolated from *G. lucidum* previously. PL-3 was closely similar with the structure of Ganoderma B isolated by Tomoda et al. (1986), and PL-4 was similar with the polysaccharide GF3 characterized by Liang and Zhang (1993) except for the exist of a small amount of 1,6-linked mannosyl residues instead of 1,6-linked galactosyl residues. The polysaccharides all showed immunoactivating properties and PL-1 was more effective than PL-3 and PL-4. The pharmacological results obtained may help in understanding the use of the fruit bodies of *G. lucidum* in the oriental countries.

3. Experimental

3.1. Materials

The fruiting bodies of *G. lucidum* (Fr.) Karst were cultivated and collected in Shanxi Province of the People's Republic of China. The fungus was identified by X.-L. Wang by comparison with a voucher specimen (No. 317), previously deposited in the herbarium of the Phytochemistry Department of the Shanghai Institute of Materia Medica, Academia Sinica, Shanghai, People's Republic of China.

Concanavalin A (ConA) and lipopolysaccharide (LPS) were obtained from Sigma and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was from Fluka. Medium RPMI 1640 was purchased from Gibco Laboratories.

3.2. General

The ^{1}H, ^{13}C and 2D NMR spectra were recorded on a Brüker AM-400 and DRX-400 NMR spectrometer equipped with a dual probe in the FT mode at room temperature. The DEPT experiment was carried out using a polarization transfer pulse of 135. Specific rotations were determined on a Perkin-Elmer 241M digital polarimeter using water as solvent. IR spectra (KBr or Nujol pellets) were obtained with a Perkin-Elmer 591B spectrophotometer. HPSEC was performed with a Waters instrument fitted with GPC software, using a Waters 2410 RI detector. GC was done with a Shimadzu-9A apparatus equipped with a 5% OV 225/AW-DMC-Chromosorb W column (2.5 m×3 mm). GC–MS was performed with a Finnigan MD-800 instrument fitted with HP-1 column, EI 70 eV. ESI–MS spectra were obtained with a VG Quattro MS/MS spectrometer.

3.3. Isolation and purification

The air-dried *G. lucidum* fruit bodies (4 kg), previously defatted with 95% alcohol, were extracted with 20 vols. hot water at 95–100 °C. The aq. extract was treated with trichloroacetic acid to remove proteins, and dialyzed against tap water for 2 days and distilled water for 1 day (molecular weight cut off 3000–5000). The retentate was concd. to a small vol. and treated with 4 vols. of EtOH. The resulting ppt. was isolated by centrifugation and then washed sequentially with EtOH and acetone. The crude product was vacuum-dried at 40 °C and gave a brownish power (PL, yield: 3.8%). A portion (8.0 g) of this product was fractionated on DEAE–cellulose column (50×10 cm) eluted with H_2O and followed stepwise by 0.05, 0.2 and 0.5 M NaCl solutions. Fractions were analyzed for total carbohydrate content and peaks for PL-1, PL-2, PL-3 and PL-4 (Fig. 1) were pooled, dialyzed, concd. and freeze-dried. Except for PL-2, each fraction was applied to a Sephacryl S-300 HR (or S-200 HR) column (100×2.6 cm), eluted with 0.2 M NaCl. Fractions were collected according to the carbohydrate profile detected by a RI detector, concd., dialyzed and lyophilized to yield PL-1 (136 mg), PL-3 (107 mg) and PL-4 (35 mg).

Table 4
Effects of PL-1 on lymphocyte proliferation, antibody production, serum IgG and complement (C3) levels in mice.[a] The mice were given samples by intraperitoneal injection at a dose of 25 mg/kg for 4 days

Spleen index (mg/g)	IgG (mg/ml)	C3 (mg/ml)	Antibody A_{520}		Lymphocytes	
					T (A_{570})	B (A_{570})
Control	5.02±1.60	17.3±1.50	2.08±0.35	1.11±0.02	0.55±0.02	0.60±0.04
PL-1	7.16±1.60	18.6±1.73	2.23±0.12	1.29±0.01**	0.78±0.01***	0.86± 0.02**

[a] Results are presented as mean±s.d. ($n=7$).**$P<0.01$ and ***$P<0.001$, significant difference from the control.

3.4. Homogeneity and M_rs

Determinations were carried out using by HPSEC under an Ultrahydrogel™ 1000 column, eluted with 0.001 M NaOH at a flow rate of 0.5 ml/min, and the data were analyzed with GPC software. The column was calibrated with standard T-series Dextran (T-500, T-110, T-80, T-70, T-40 and T-9.3). All samples were prep. as 0.1% (w/v) solns. and 20 μl of soln. was analyzed in each run.

3.5. Compositional and D, L-configuration analyses

The sugar compositions of PL-1, PL-3 and PL-4 were determined by TLC of the acid hydrolysates and by GC analysis of the alditol acetates derivatives as described by Blakeney et al. (1983).

The absolute configurations of the sugars were identified by GC analysis of their TMSi (−)-2-butylglycosides derivatives (Gerwig et al., 1978).

3.6. Glycosyl linkage composition analyses

The native polysaccharides and polymers generated by partial acid hydrolyses were methylated three or four times by the modified NaOH–DMSO method (Needs and Selvendran, 1993). The per-methylated product was hydrolyzed, reduced, acetylated and then analyzed by GC–MS as previously described (Bao et al., 2001). The partially methylated alditol acetates were identified by their fragment ions in EI–MS and by relative times in GC, and the molar ratios were estimated from the peak areas and response factors (Sweet et al., 1975).

3.7. Partial acid hydrolyses of PL-1

PL-1 (200 mg) was dissolved in 0.05 M TFA (100 ml) and kept at 100 °C for 1 h. After cooling, conc. and dialysis, the retentate was lyophilized to give a degraded polymer (PL-1-A, 162 mg). The glycosyl residue composition of PL-1-A was determined by GC analysis. The dialysate was isolated on a Sephadex G-10 column (100×1.6 cm). A portion of PL-1-A (8 mg) was methylated and its glycosyl linkage composition was determined as described above. The remaining portion (146 mg) was further hydrolyzed with 0.1 M TFA at 100 °C for 1.5 h. A depolymerized product (PL-1-B, 60 mg) was obtained and the oligosaccharide fraction was separated by Sephadex G-10 chromatography. According to the carbohydrate profile of the column, the oligosaccharide fractions were pooled and lyophilized, respectively, and their glycosyl residue compositions were determined by GC as alditol acetates.

3.8. Acetolysis of PL-1

PL-1 (50 mg) was acetylated by a mixture of acetic anhydride and pyridine (1:1, v/v) at 100 °C for 24 h. After the excess acetic hydride and pyridine were completely removed, the resulting product was treated with HAc–Ac_2O–conc. H_2SO_4 (10:10:1, v/v/v) at 40 °C for 8 h (Vaishnav et al., 1997). The acetolysate was de-*O*-acetylated by treatment with 0.2 M NaOMe in MeOH, and the soln. of oligosaccharides was deionised with Dowex 50 (H^+) resin. The resulting solution was applied to a Sephadex G-10 column and the oligosaccharide fractions were collected according to the carbohydrate profile of the eluants.

3.9. Periodate oxidation of PL-3 and PL-4

PL-3 (50 mg) and PL-4 (50 mg) were dissolved in 0.02 M $NaIO_4$ (50 ml) and kept in the dark at 5 °C, respectively (Vaishnav et al., 1997; Tomoda et al., 1986), with the absorption at 224 nm monitored every day. The oxidation was completed after 7 days and ethylene glycol (0.6 ml) was added to the solution. Consumption of $NaIO_4$ was measured by a spectrophotometric method (Dixon and Lipkin, 1954) and HCOOH production by titration with 0.01 M NaOH. The reaction mixtures of oxidative PL-3 and PL-4 were reduced with $NaBH_4$, dialyzed and then mildly hydrolyzed with 0.2 M TFA at 40 °C for 24 h. The resulting solution was neutralized, dialyzed and the retentate was then lyophilized. The glycosyl residue composition of the resulting product from the oxidation of PL-4 was analyzed by GC as alditol acetates.

3.10. Immunological assays

Immunological activity of PL-1, PL-3 and PL-4, obtained from the fruiting bodies of *G. lucidum* was

assessed by measuring the ConA or LPS induced T- and B-lymphocyte proliferations in vitro and in mice. Serum IgG and C-3 levels were measured in mice too.

Inbred ICR (♀) mice, three months old, weighing 20 ± 2 g, were obtained from Shanghai Experimental Animal Laboratory, Chinese Academy of Sciences (certificate No. 133). For the tests in vitro, various dilutions of the polysaccharide samples (1–100 μg/ml) were incubated with mouse splenocytes in the presence of mitogen ConA (5.0 μg/ml) or LPS (20 μg/ml). After incubation for 44 h at 37 °C in a humidified 5% CO_2 atmosphere, T- and B-lymphocyte proliferations were assayed by the MTT method as reported previously (Heek et al., 1985; Zhou et al., 1986).

For the in vivo tests, 14 mice were divided randomly into 2 groups: normal saline (control group), PL-1 25 mg/kg. The mice were given samples by intraperitoneal injection for 4 successive days, respectively. Mice were sacrificed on day 5 and their spleens were removed, minced and passed through a sterilized ion mesh (200 mesh) to obtain single cell suspension. Erythrocytes in the cell mixture were destroyed by the rapid addition of H_2O. Finally, the cells were suspended to 5×10^6 cells/ml in an RPMI 1640 medium. The lymphocyte proliferation and antibody production of spleen cells were measured by the MTT method or quantitative hemolysinspectrophotomic assay as described in previous reports (Xiang, and Li, 1993). Serum IgG and complement (C-3) levels were determined by single immunodiffusion method described previously (Li et al., 1990). The diameters of samples on a rabbit anti-mouse serum plate diffusion ring were measured.

The data were represented as mean±s.d. Student's *t*-test for unpaired observations between control and tested samples was carried out to identify statistical differences.

Acknowledgements

The authors are grateful to the Shanghai Institutes for Biological Sciences and Shanghai Committee of Science and Technology for financial support. We also express our thanks to Shanghai Green Valley company (Shanghai, People's Republic of China) for providing the dried *G. lucidum* fruiting bodies.

References

Bao, X.F., Fang, J.N., 2001. Isolation and structural determination of a glucan from spores of *Ganoderma lucidum*. Acta Botanica Sinica 43, 312–315.

Bao, X.F., Liu, C.P., Fang, J.N., Li, X.Y., 2001. Structural and immunological studies of a major polysaccharide from spores of *Ganoderma lucidum* (Fr.) Karst. Carbohydrate Research 332, 67–74.

Blakeney, A.B., Harris, P.J., Henry, R.J., Stone, B.A., 1983. A simple and rapid preparation of alditol acetates for monosaccharide analysis. Carbohydrate Research 113, 291–299.

Bubb, W.A., Urashima, T., Fujiwara, R., Shinnai, T., Ariga, H., 1983. Structural characterization of the exopolysaccharide produced by *Streptococcus thermophilus* OR 901. Carbohydrate Research 301, 41–50.

Dixon, J.S., Lipkin, D., 1954. Spectrophotometric determination of vicinal glycols: application to the determination of ribofuranosides. Analytical Chemistry 26, 1092–1093.

Franz, G., 1989. Polysaccharides in pharmacy: current applications and future concepts. Planta Medica 55, 493–497.

Furusawa, E., Chou, S.C., Furusawa, S., Hirazami, A., Dang, Y., 1992. Antitumor activity of *Ganoderma lucidum*, an edible mushroom, on intraperitoneally implanted Lewis Lung Carcinoma in synergenic mice. Phytotherapy Research 6, 300–304.

Gerwig, G.J., Kamerling, J.P., Vliegenthart, J.F.G., 1978. Determination of the D and L configuration of neutral monosaccharides by high-resolution capillary G.L.C. Carbohydrate Research 62, 349–357.

Ghosh, T.K., Rao, C.C., 1981. Some structural features of the D-glucan from the seed of *Mirabilis jalapa*. Carbohydrate Research 90, 243–250.

Heek, K., Reimann, J., Kabelitz, D., Hardt, C., Wagner, H., 1985. A rapid colorimetric assay for the determination of IL-2-producing helper T cells frequencies. Journal of Immunological Methods 77, 237–246.

Hikino, H., Konno, C., Mirin, Y., Hayashi, T., 1985. Isolation and hypoglycemic activity of Ganoderma A and B, glycan of *Ganoderma lucidum* fruit bodies. Planta Medica 51, 339–340.

Karlsson, C., Jansson, P.-E., Widmalm, G., Sorensen, U.B.S., 1997. Structural elucidation of the capsular polysaccharide from *Streptococcus pneumoniae* type 18B. Carbohydrate Research 304, 165–172.

Li, X.Y., Wang, J.F., Zhu, P.P., Liu, L., Ge, J.B., Yang, S.X., 1990. Immune enhancement of a polysaccharide peptide isolated from *Corrolus versicolor*. Acta Pharmacologica Sinica 11, 542–545.

Liang, Z.Y., Zhang, Y.S., 1993. Isolation purification and structural investigation of water soluble polysaccharide GF3 from *Ganoderma tsugae* murr in Changbai Mountain. Acta Biochimica. et Biophysica. Sinica. 25, 59–62.

Miyazaki, T., Nishijima, M., 1981. Structural examination of a water soluble, antitumor polysaccharide of *Ganoderma lucidum*. Chemical and Pharmaceutical Bulletin 29, 3611–3616.

Needs, P.W., Selvandran, R.R., 1993. Avoiding oxidative degradation during sodium hydroxide/methyl idodide-mediated carbohydrate methylation in dimethyl sulfoxide. Carbohydrate Research 245, 1–10.

Paramonov, N.A., Parolis, L.A.S., Parolis, H., Boan, I.F., Anton, J., Rodriguez-Valera, F., 1998. The structure of the exocellular polysaccharide produced by the Archaeon *Haloferax gibbonsii* (ATCC 33959). Carbohydrate Research 309, 89–94.

Shiao, M.S., Lee, K.R., Lin, L.J., Wang, C.T., 1994. Natural products and biological activities of the Chinese medical fungus, *Ganoderma lucidum*. In: Ho, C.T., Osawa, T., Huang, M.T., Rosen, R.T. (Eds.), Food Phytochemicals for Cancer Prevention. II: Teas, Spices and Herbs. American Chemical Society, Washington DC, pp. 342–354.

Sweet, D.P., Shapiro, R.H., Albersheim, P., 1975. Quantitative analysis by various GLC response-factor theories for partially methylated and partially ethylated alditol acetates. Carbohydrate Research 40, 217–225.

Tomoda, M., Gonda, R., Kasahara, Y., Hikino, H., 1986. Glycan structures of *Ganoderma lucidum* B and C, hypoglycemic glycans of *Ganoderma lucidum* fruit bodies. Phytochemistry 25, 2817–2820.

Tsumuraya, Y., Misaki, A., 1979. Structure of the water-insoluble α-D-glucan of *Streptocossus salivarius* HHT. Carbohydrate Research 74, 217–225.

Vaishnav, V.V., Bacon, B.E., O'Neill, M., Cherniak, R., 1997. Structural Characterization of the galactoxylomannan of *Cryptococcus neoformans* Cap 67. Carbohydrate Research 306, 315–330.

Wang, S.Y., Hsu, M.L., Hsu, H.C., Tzeng, C.H., Lee, S.S., Shiao, M.S., Ho, C.K., 1997. The anti-tumor effect of *Ganoderma lucidum* is mediated by cytokines reduced from activated macrophages and T lymphocytes. International Journal of Cancer 70, 699–705.

Xiang, D.B., Li, X.Y., 1993. Antitumor activity and immuno-potentiating actions of *Achyranthes bidentata* polysaccharides. Acta Pharmacologica Sinica 14, 556–561.

Zhou, D.H., Shen, Y.S., Zhao, M.R., 1986. The application of MTT colorimetric assay to measure the proliferation of lymphocytes and the activity of rat/mouse IL-2. Chinese Journal of Immunology 2, 39–46.