JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Isolation, Structure Characterization, and Immunomodulating Activity of a Hyperbranched Polysaccharide from the Fruiting Bodies of *Ganoderma sinense*

Xiao-Qiang Han,[†,#,∥] Ben Chung Lap Chan,[†,∥] Cai-Xia Dong,[§] Yin-Hua Yang,[‡] Chun-Hay Ko,[†] Grace Gar-Lee Yue,[†] Dan Chen,[⊗] Chun-Kwok Wong,[†,⊥] Clara Bik-San Lau,[†] Peng-Fei Tu,*[,#] Pang-Chui Shaw,[‡] Kwok-Pui Fung,[†] Ping-Chung Leung,[†] Wen-Luan Hsiao,[§] and Quan-Bin Han*[,†,§]

[†]State Key Laboratory of Phytochemistry and Plant Resources in West China, Institute of Chinese Medicine, The Chinese University of Hong Kong, Shatin, NT, Hong Kong SAR, China

[§]School of Chinese Medicine, Hong Kong Baptist University, Hong Kong SAR, China

[#]State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University Health Science Center, Beijing 100191, China

[‡]School of Life Sciences and Centre for Protein Science and Crystallography, The Chinese University of Hong Kong, Shatin, NT, Hong Kong SAR, China

[⊗]Department of Pharmacology, School of Basic Medical Sciences, Tianjin Medical University, Tianjin 300070, China

[⊥]Department of Chemical Pathology, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, NT, Hong Kong SAR, China

ABSTRACT: A polysaccharide (GSP-6B) with a molecular mass of 1.86×10^6 Da was isolated from the fruiting bodies of *Ganoderma sinense*. Chemical composition analysis, methylation analysis, infrared spectroscopy, and nuclear magnetic resonance spectroscopy were conducted to elucidate its structure. GSP-6B contains a backbone of (1→6)-linked-*β*-D-glucopyranosyl residues, bearing branches at the *O*-3 position of every two sugar residues along the backbone. The side chains contain (1→4)-linked-*β*-D-glucopyranosyl residues, (1→3)-linked-*β*-D-glucopyranosyl residues, and nonreducing end *β*-D-glucopyranosyl residues. An in vitro immunomodulating activity assay revealed that GSP-6B could significantly induce the release of IL-1*β* and TNF-*α* in human peripheral blood mononuclear cell (PBMC) and showed no toxicity to either PBMC or a human macrophage cell line THP-1. GSP-6B could also activate dendritic cells (DC) by stimulating the secretion of IL-12 and IL-10 from DC.

KEYWORDS: *Ganoderma sinense, polysaccharide, immunological activity, cytokine, dendritic cell*

INTRODUCTION

Lingzhi is one of the most well-known functional foods and has been popularly used in Asian countries for a long time. The general name of *Ganoderma* species was cited as early as 100 B.C. in China.[1] Although this genus is a big family, now the name Lingzhi usually means a single species *Ganoderma lucidum*, which contains various phytochemicals, such as triterpenoids, steroids, alkaloids, nucleosides, proteins, and polysaccharides.[1] Polysaccharides are believed to be the major active ingredient, showing antioxidant activities,[2−4] immunomodulating activities,[5,6] and antitumor activities.[7−9] The major bioactive polysaccharide is 1,3-linked *β*-D-glucopyranosyl with 1−15 units of 1,6-linked *β*-D-glucopyranosyl side chains.[1]

Besides *G. lucidum*, *Ganoderma sinense* is another official species of Lingzhi in China. It also exhibits multiple pharmacological effects, such as immunomodulating, antitumor, hepatoprotecting, antioxidant, and cholesterol-lowering activities.[1,10−13] Recent studies indicated that these two food materials might be quite different. Ganodermic acids, often reported as anticancer compounds, are major components of the ethanol extract of *G. lucidum*, but little or even none at all is found in *G. sinense*.[1,14] It is suggested that significant difference might also exist in their polysaccharide profile. Compared to *G. lucidum*, of which the polysaccharide components have been well studied in terms of chemical structures and bioactivities, little is known about the chemistry of purified polysaccharides from *G. sinense*. Because the polysaccharide crude extract of *G. sinense* showed significant immunomodulating activity,[15−19] and the structural characterization is essential for demonstration of the pharmacological mechanism and quality control, we start an exploration of chemistry and immunological activities of purified polysaccharides of *G. sinense*. Herein we report the isolation, structure elucidation, and in vitro immunomodulating activity of a novel hyperbranched polysaccharide from the fruiting bodies of *G. sinense*.

EXPERIMENTAL PROCEDURES

Materials and Chemicals. Dried fruiting bodies of *G. sinense* were purchased from a herbal store in Hong Kong and authenticated by

Received: December 20, 2011
Revised: April 12, 2012
Accepted: April 12, 2012
Published: April 13, 2012

ACS Publications © 2012 American Chemical Society

dx.doi.org/10.1021/jf205056u | J. Agric. Food Chem. 2012, 60, 4276−4281

Professor Zhu-Liang Yang at Kunming Institute of Botany, Chinese Academy of Sciences. The voucher specimen is deposited at the Institute of Chinese Medicine, the Chinese University of Hong Kong, with voucher specimen no. 2010−3271. DEAE-Sepharose CL-6B was purchased from Amersham Pharmacia Biotech (Uppsala, Sweden). Sephacryl S-300 and 400 HR were purchased from Pharmacia Biotech (Piscataway, NJ, USA). Standard monosaccharides, T-series dextrans, trifluoroacetic acid (TFA), dimethyl sulfoxide (DMSO), lipopolysaccharide (LPS), and RPMI 1640 medium were purchased from Sigma (St. Louis, MO, USA). The human blood samples were supplied by Hong Kong Red Cross Blood Transfusion Service, and the human macrophage cell line THP-1 was purchased from American Type Culture Collection (ATCC; Manassas, VA, USA). ELISA test kits for IL-1β, IL-10, IL-12, and TNF-α were purchased from BD Pharmingen Corp. (San Diego, CA, USA).

Analytical Methods. The UV−vis spectra were tested on a Beckman DU650 ultraviolet and visible spectrophotometer. Gas chromatography−mass spectrometry (GC-MS) tests for analysis of the sugar composition were performed on a Shimadzu QP-2010 instrument equipped with a DB-5 column (30 m × 0.25 mm × 0.25 μm) and a quadrupole rods mass detector (225 °C); the column temperature was increased from 140 to 225 °C at a rate of 2 °C/min and then kept at 225 °C for 5 min. GC-MS tests for methylation analysis were measured with a DB-225 column (30 m × 0.25 mm × 0.25 μm) and at temperatures from 40 to 225 °C programmed at 40 °C/min and then kept at 225 °C for 30 min. The Fourier transform infrared spectroscopy (KBr pellets) was recorded on SPECORD in a range of 400−4000 cm^{-1}.

Extraction and Isolation. Dried fruiting bodies of *G. sinense* (1.0 kg) were extracted twice by distilled water (2 L × 2) at 100 °C for 1 h. The combined water extracts were collected and concentrated to 300 mL under a reduced pressure. After centrifugation at 3000 rpm for 10 min, the supernatant was concentrated and precipitated with 95% ethanol (4 times of volume) at 4 °C overnight. The sediment was then dissolved in an appropriate volume of distilled water and intensively dialyzed for 2 days against distilled water (cutoff M_w 7000 Da). After concentration, the retentate was deproteinized with Sevage reagent ($CHCl_3$/BuOH = 4:1, v/v) for 15 min and the procedure was repeated seven times. Finally, the extracts were centrifuged to remove insoluble material, and the supernatant was freeze-dried as the crude *G. sinense* polysaccharides (coded GSP).

A portion of the crude polysaccharides (2.2 g) dissolved in water (50 mL) was loaded on a DEAE-Sepharose CL-6B column (5.0 × 70.0 cm) and eluted with a six-step gradient with distilled water, 0.1 M sodium chloride (NaCl), 0.3 M NaCl, 0.5 M NaCl, 1.0 M NaCl, and 0.2 M sodium hydroxide (NaOH). The elution was monitored using the phenol−sulfuric acid method. The 0.2 M NaOH fraction was collected as a major fraction, neutralized by 0.2 M hydrochloric acid, dialyzed, lyophilized, and purified by gel permeation chromatography on Sephacryl S-300 and 400 HR eluting with water, to afford a purified polysaccharide (GSP-6B).

Estimation of Homogeneity and Apparent Molecular Mass. The homogeneity and molecular mass of GSP-6B were estimated on a Waters Acquity UPLC system equipped with a TSK-SW4000 column and an evaporative light scattering detector (ELSD). Ten microliters of sample solution (1.0 mg/mL) was injected each run, with water as the mobile phase at a flow rate of 0.3 mL/min. The linear regression was calibrated with T-series dextran standards (M_w 2000, 670, 410, 270, 150, 80, 50, 12, 5, and 1 kDa).

Monosaccharide Composition Analysis. The identification and quantification of the monosaccharides of GSP-6B (10 mg) were achieved by GC-MS analysis. GSP-6B (10 mg) was hydrolyzed with 2 M trifluoroacetic acid (TFA) at 100 °C for 3 h. The monosaccharides were analyzed by GC-MS after complete conversion into their acetylated derivation according to the method of Lawrence and Lyengar.[20]

Methylation Analysis. GSP-6B (10 mg) was methylated three times according to the method described in the literature.[21] 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.[22] The partially methylated alditol acetates were analyzed by GC-MS.

NMR Analysis. GSP-6B (30 mg) was dried in a vacuum over P_2O_5 for 72 h and then exchanged with deuterium by lyophilization with D_2O three times. After that, the sample was put in a 5 mm NMR tube and dissolved in 1.0 mL of 99.96% D_2O. All spectra were obtained at 298 K on a Bruker Avance 700 MHz NMR spectrometer equipped with a TCI cryoprobe. Tetramethylsilane (TMS) was used as external standard in the ^{13}C NMR test, and D_2O was used as internal standard in the ^{1}H NMR test.

Partial Hydrolysis. GSP-6B (20 mg) was partially hydrolyzed with 0.1 M TFA at 60 °C for 10 h. The hydrolysate was then dialyzed against distilled water for 24 h in a dialysis bag with a 7000 Da cutoff. The residues in the dialysis bag was collected and coded GSP-6B-in.

Amino Acid Analysis. GSP-6B (10 mg) was hydrolyzed under vacuum at 110 °C in 6 M hydrochloric acid for 24 h. The hydrolysis products were determined with a Hitachi 835-50 amino acid analysis analyzer, with a group of standard amino acids as the markers. The content of each kind of amino acids was calculated using the standard curves.

Measurement of Immunomodulating Activity. *Effect of GSP-6B on Cytokine Secretion of Human Peripheral Blood Mononuclear Cell (PBMC) .*[23] The fresh buffy coat was diluted with phosphate-buffered saline at a ratio of 1:1. The diluted sample (20 mL) was put in a 50 mL centrifuge tube together with an equal volume of Ficoll−Plaque Plus solution. The tube was then centrifuged at 800*g* for 20 min at 18 °C. The supernatant was discarded, and the PBMCs were resuspended in 4 mL of RPMI 1640 medium plus 10% fetal bovine serum (FBS). The cell number was counted, and the viability of the cell was checked by trypan blue exclusion assay.

The isolated PBMCs were seeded in a 96-well flat-bottom microplate and incubated with GSP-6B, LPS, or dextran (0.00003−100 μg/mL). After 24 h of treatment, concentrations of IL-1β and TNF-α in culture supernatant were determined using ELISA kits (BD Pharmingen Corp.). Polyminxin B (PMB), which is a specific inhibitor of LPS, was added in the sample of GSP-6B to exclude the influence of LPS.

XTT Proliferation Assay .[24] The cytotoxicity of GSP-6B on PBMCs and a human macrophage cell line (THP-1) was determined by XTT proliferation assays (Roche) according to the manufacturer's instructions. In brief, 2 × 10^5 THP-1 cells or PBMCs were seeded into flat 96-well plates. The cells were cultured in the presence of GSP-6B, LPS, or dextran at different concentrations for 72 h. After the incubation period, 50 μL of XTT/phenazinemethosulfate solution (20 μM) was added to each well for a further 4 h of incubation at 37 °C. Finally, the colorimetric changes of each well were measured at a wavelength of 490 nm. The toxicity represents the ratio of the OD of a well in the presence of tested compounds with the OD of the drug-free control wells. A cellular viability of at least 90% was considered to indicate a nontoxic compound.

Effect of GSP-6B on Production of IL-10 and IL-12 in Human Dendritic Cell (DC) .[25] For generation of human monocyte derived dendritic cells (DC) from PBMC, monocytes were positively purified from PBMCs by attachment method. Cells were cultured at 2 × 10^6 cells/mL in RPMI/10% FBS medium supplemented with granulocyte-GM-CSF (50 ng/mL) and IL-4 (40 ng/mL) for 6 days. The immature DC were then harvested and incubated for 2 days by adding GSP-6B, LPS, or dextran (0.00003−100 μg/mL). Supernatants were collected, and the concentrations of IL-10 and IL-12 were determined by ELISA.

Statistical Analysis. All experiments were repeated at least three times. Results are presented as the mean ± the standard error of the mean (SEM). Comparison of the data was performed using the single-factor ANOVA test. Significance was defined as a p value of <0.05.

RESULTS AND DISCUSSION

A water-soluble polysaccharide coded GSP-6B was isolated from the fruiting bodies of *G. sinense*. It exhibited a single and symmetrical peak in the HPLC-GPC analysis, which indicated

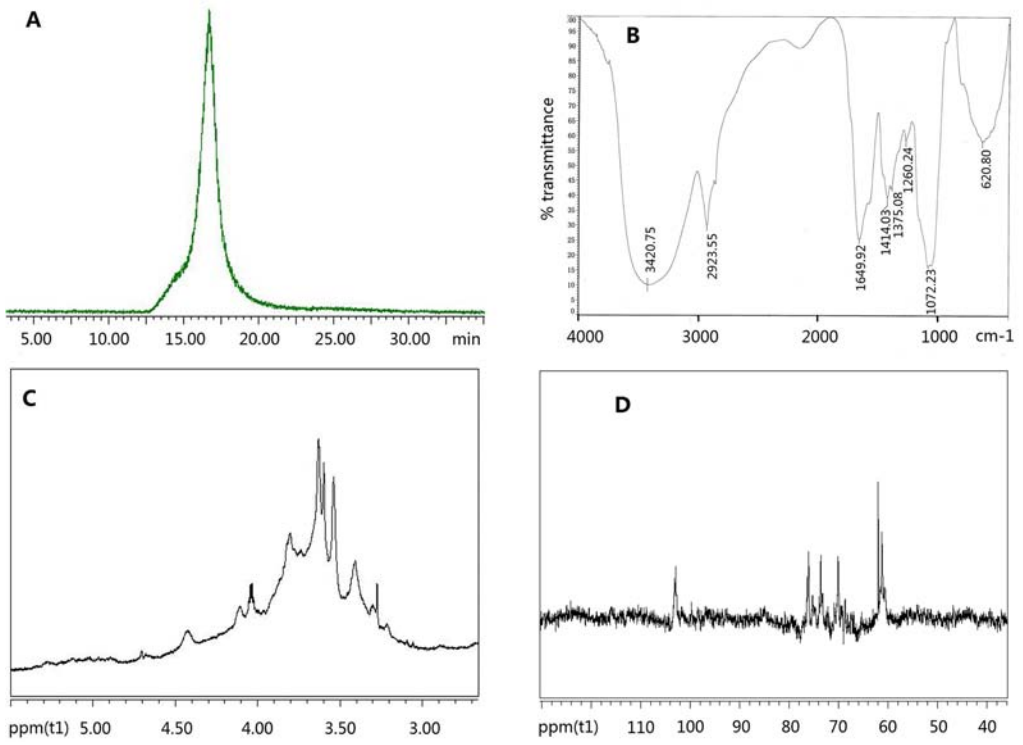

Figure 1. UPLC chromatogram and FT-IR and NMR spectra of the polysaccharide GSP-6B. (A) UPLC chromatogram. For molecular weight evaluation a Waters Acquity UPLC system equipped with a TSK-SW4000 column and an evaporative light scattering detector (ELSD) was used, with distilled water as mobile phase at a flow rate of 0.3 mL/min. The molecular mass was calculated referring to a series dextran molecular standards. (B) FT-IR spectrum. The Fourier transform infrared spectroscopy (KBr pellets) was recorded on SPECORD in a range of 400−4000 cm^{-1}. (C) ^{1}H NMR spectrum. The ^{1}H NMR spectrum of GSP-6B was obtained on a Bruker AM 700 spectrometer (700 MHz) with a dual probe in the FT mode at room temperature. D_2O was used as internal standard. (D) ^{13}C NMR spectrum. The ^{13}C NMR spectrum of GSP-6B was obtained on a Bruker AM 700 spectrometer (175 MHz) with a dual probe in the FT mode at room temperature. TMS was used as external standard.

its homogeneity based on the distribution of molecular weight. Its molecular mass was determined as 1.86×10^6 Da according to the retention time (Figure 1A). GC-MS analysis indicated that GSP-6B was mainly composed of glucose, with trace amounts of galactose and mannose. Its protein content was determined as 10.13% by analysis of the amino acids, as listed in Table 1. Because the deproteinization process was repeated for more than seven times, it is supposed that these amino acids mainly remained on the sugar chain of GSP-6B by covalent bonds. The existence of these amino acids might enhance the flexibility of the polysaccharide chain and further make it more easily identified by the receptors on the surface of lymphocyte[26−28] In the FT-IR spectrum of GSP-6B, the strong band at 3420.8 cm^{-1} was attributed to the hydroxyl stretching vibration of the polysaccharide, and that at 2923.6 cm^{-1} was due to the C−H stretching vibration absorption. The absence of any bands at 1735 cm^{-1} confirmed that this fraction did not contain uronic acidic, and the characteristic bands at 1000−1100 cm^{-1} suggested the presence of pyranose form of the glucosyl residue (Figure 1B).[29−31]

Table 1. Amino Acid Composition Analysis Result of the Polysaccharide (GSP-6B) Isolated from the Fruiting Bodies of *Ganoderma sinense*[a,b]

amino acid	content (μg/mg)	amino acid	content (μg/mg)
aspartic acid	13.42	leucine	13.45
threonine	6.9	tyrosine	2.08
serine	5.65	phenylalanine	5.94
glutamic acid	12.77	lysine	4.24
glycine	7.34	histidine	1.33
alanine	8.2	arginine	3.23
valine	7.94	proline	3.96
methionine	0.71		
isoleucine	4.14	total	101.3

[a]All of the result were tested by a Hitachi automatic amino acid analyzer. [b]The molecular ratio of amino acids was calculated directly in accordance with the peak areas.

The glycosidic linkage types of sugar residues were determined by GC-MS analysis in which mainly five peaks were observed, corresponding to five partially methylated alditol acetates (1,5,6-triacetyl-2,3,4-tri-*O*-methyl glucitol, 1,3,5,6-tetra-acetyl-2,4-di-*O*-methyl glucitol, 1,3,5-triacetyl-2,4,6-tri-*O*-methyl glucitol, 1,4,5-triacetyl-2,3,6-tri-*O*-methyl glucitol, and 1,5-diacetyl-2,3,4,6-tetra-*O*-methyl glucitol) in the molar ratio of nearly 2:2:4:3:2 (Table 2). The above analysis suggested that GSP-6B should contain five glucosidic linkage forms: (a) (1→6)-linked glucosyl (residue A), (b) (1→3,6)-linked glucosyl (residue B), (c) (1→3)-linked glucosyl (residue C), (d) (1→4)-linked glucosyl (residue D), and (e) nonreducing terminal (residue E), indicating that GSP-6B is a branched polysaccharide.

To establish the residue sequence, GSP-6B was partially hydrolyzed using TFA (0.1 M). Methylation analysis revealed (1→6)-linked glucosyl residues as the major degradation product, indicating that the backbone of GSP-6B was 1,6-glc*p* with branches at the *O*-3 position. The disappearance of (1→

Table 2. GC-MS Data for the Methylated Sugar Moieties of GSP-6B[a,b]

residue	methylated sugar	type of linkage	molar ratio
A	2,3,4Me3-Glc	1,6-linked Glc*p*	2
B	2,4-Me2-Glc	1,3,6-linked Glc*p*	2
C	2,4,6-Me3-Glc	1,3-linked Glc*p*	4
D	2,3,6-Me3-Glc	1,4-linked Glc*p*	3
E	2,3,4,6-Me4-Glc	terminal Glc*p*	2

[a]The results were tested on a DB-5 GC-MS column and a DB-225 column. [b]All of the sugar residues were primarily identified by their MS spectrum and further confirmed by their relative retention time to 2,3,4,6-Me4-Glc.

3)-linked glucosyl and (1→4)-linked glucosyl residues indicated that they were parts of the side chains.

Only one set of anomeric signals (δ 4.42 and 102.5) was observed in the 1D NMR spectrum of GSP-6B, which confirmed that all of the residues are of β-configuration (Figure 1C,D). Compared to the normal shift value at δ 78, the signals at δ 84.0 were attributable to C-3 of both residues B and C, which were significantly down-shifted due to the α effect of glycosidation.[28,29] Similarly, the signal at δ 79.1 was assigned to C-4 of residue D and that at δ 69.2 to C-6 of residues A and B. These assignments were confirmed by the results of methlyation analysis. According to the above-mentioned, GSP-6B was deduced to be a polysaccharide having a backbone structure of (1→6)-linked-β-D-glucopyranosyl residues, with substitution at the *O*-3 position on average two residues, and the branches were composed of (1→3)-linked-β-D-glucopyranosyl residues, (1→4)-linked-β-D-glucopyranosyl, and terminated with nonreduced end β-D-glucopyranosyl residues. This is the first example of highly branched polysaccharide with a backbone of (1,6)-linked Glc*p* from the fruiting bodies of the mushroom of *Ganoderma*, whereas the most reported are (1,3)-linked glucans.

As a crucial event in the activation cascade of both cellular and humoral immune responses, stimulation of human PBMC was primarily selected to evaluate the immunological activity of GSP-6B. In cultured PBMC, GSP-6B was found to induce the release of two pro-inflammatory cytokines, IL-1β and TNF-α, in a dose-dependent manner from 0.00003 to 100 μg/mL (Figure 2A,B). Dextran and LPS were used as negative and positive control, respectively. Moreover, GSP-6B was shown to be nontoxic to PBMC in XTT studies (Figure 3A).

Among PBMC, IL-1β and TNF-α are mainly secreted from monocytes. Monocytes and macrophages are phagocytes in the innate immune system with the functions of bacteria destruction, antigen presentation, tumor cell destruction, and tissue wound healing. It is also the first group of immune cells that interact with the exogenous polysaccharides. Macrophages in the intestine interact with those polysaccharide undigested by the stomach digestive ferment, identify them, and then engulf them. Macrophages will then degrade these macromolecular polysaccharides into multismall oligosaccharides, which will be further presented to the T and B lymphocytes for adaptive immune response.[30−33] By using a human macrophage cell line THP-1, we further demonstrated that GSP-6B was also nontoxic to THP-1 (Figure 3B).

As one of the potent and professional antigen-presenting cells, dendritic cells can effectively take up, process, and present specific antigens, which play a central role in immune response by bridging the innate and acquired immunity. For cytokines secreted from activated DCs, IL-12 is a crucial factor for modulating Th1 response and IL-10 is a potent anti-inflammatory mediator. IL-10 induction was TLR ligand selective, in that CpG DNA, imidazoquinolin, peptidoglycan, and zymosan but not LPS and poly I:C led to IL-10 production.[34] As shown in Figure 4A, GSP-6B could significantly activate DC by stimulating the secretion of IL-12 from DC from 1 to 100 μg/mL. Significant secretions of IL-10 were also observed when DC were incubated with GSP-6B (5−100 μg/mL) but not LPS (Figure 4B), suggesting that the activation of DC by GSP-6B might be TLR-2- or dectin-1-dependent. Further investigations are required for validation of this issue.

In summary, a water-soluble polysaccharide, GSP-6B, was isolated from the fruiting bodies of a medicinal mushroom, *G. sinense*. Its structure was elucidated by chemical methods and modern spectroscopic technologies. GSP-6B is a hyperbranched polysaccharide, owning a backbone structure of (1→6)-linked-β-D-glucopyranosyl residues, which branched at the *O*-3 position on average two sugar residues. An in vitro immunomodulating activity assay revealed that GSP-6B could significantly induce the release of IL-1β and TNF-α in human PBMC in a dose-dependent manner and showed no toxicity to

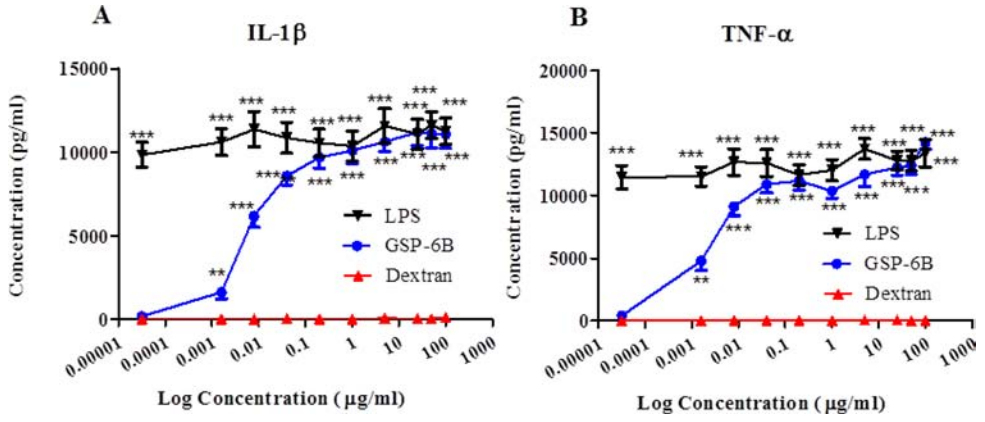

Figure 2. Effects of GSP-6B on the secretion of cytokines (A) IL-1β and (B) TNF-α in PBMCs (n = 8). For the assay of cytokine secretion in PBMCs, cells were cultured with different concentrations of GSP-6B (0.00003−100 μg/mL) for 48 h, and the release of IL-10 and IL-12 was determined by ELISA, n = 8. Dextran and LPS were used as negative and positive control, respectively. Significant differences are indicated by asterisks: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

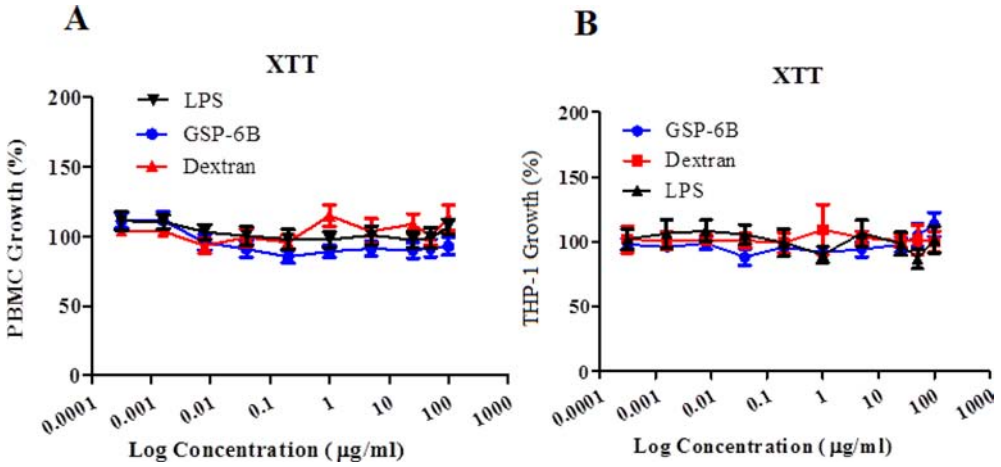

Figure 3. Effect of GSP-6B on the proliferation of (A) PBMCs and (B) human macrophage cell line THP-1. THP-1 cells or PBMCs (2×10^5 cells in RPMI with 10% fetal bovine serum) were treated with GSP-6B (0.00003−100 μg/mL) for 72 h ($n = 8$). Dextran and LPS were used as negative and positive control, respectively. Significant differences are indicated by asterisks: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

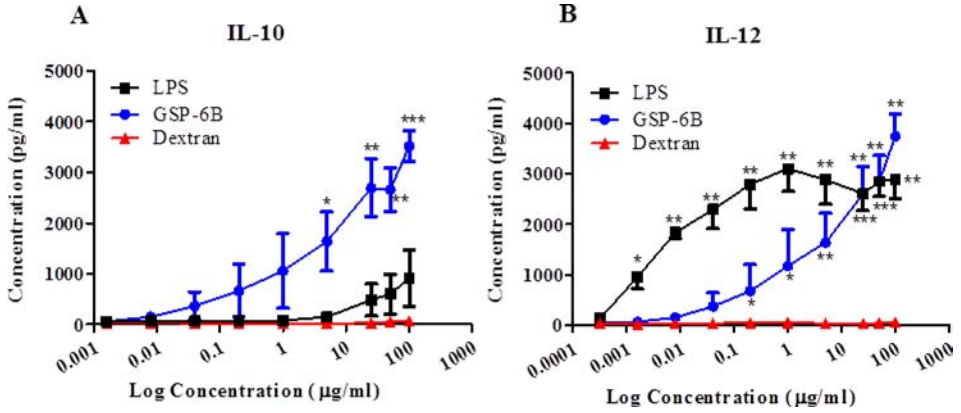

Figure 4. Effects of GSP-6B on the secretion of (A) IL-10 and (B) IL-12 on human monocyte derived dendritic cells (DC) ($n = 5$). Immature DC were cultured in RPMI with 10% FBS with different concentrations of GSP-6B (0.00003−100 μg/mL) for 48 h, and the release of IL-10 and IL-12 was determined by ELISA, $n = 5$. Dextran and LPS were used as negative and positive control, respectively. Significant differences are indicated by asterisks: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

either PBMC or a human macrophage cell line THP-1. GSP-6B could also activate DC by stimulating the secretion of IL-12 and IL-10 from DC. It was therefore suggested that GSP-6B was one of the active components of *G. sinense* and had the potential as adjuvant therapy medicines against tumors. This is the first report of the structure characterization and bioactivity of a high-purity polysaccharide from *G. sinense*.

AUTHOR INFORMATION

Corresponding Author

*(Q.-B.H.) Phone: 852-3411 2906. E-mail: simonhan@hkbu.edu.hk. (P.-F.T.) Phone: 86-010-8280 2750. E-mail: pengfeitu@bjmu.edu.cn.

Author Contributions

‖Equal contribution.

Funding

This research is funded by the Innovation and Technology Fund (ITS/311/09 and InP/108/10) of the Government of the Hong Kong Special Administrative Region and Hong Kong Baptist University (FRG2/11-12/048).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The purchase of the 700 MHz nuclear magnetic spectrometer was by a One-Off Special Equipment Grant (SEG CUHK08 and CUHK09) of the University Grants Council of Hong Kong SAR.

REFERENCES

(1) Paterson, R. R. M. Ganoderma: a therapeutic fungal biofactory. *Phytochemistry* **2006**, *67*, 1985−2001.

(2) Chen, X. P.; Chen, Y.; Li, S. B.; Chen, Y. G.; Lan, J. Y.; Liu, L. P. Free radical scavenging of *Ganoderma lucidum* polysaccharides and its effect on antioxidant enzymes and immunity activities in cervical carcinoma rats. *Carbohydr. Polym.* **2007**, *77*, 389−393.

(3) Jia, J.; Zhang, X.; Hu, Y. S.; Wu, Y.; Wang, Q. Z.; Li, N. N. Evaluation of in vivo antioxidant activities of *Ganoderma lucidum* polysaccharides in STZ-diabetic rats. *Food Chem.* **2009**, *115*, 32−36.

(4) Xu, J.; Liu, W.; Yao, W. B.; Pang, X. B.; Yin, D. K.; Gao, X. D. Carboxymethylation of a polysaccharide extracted from *Ganoderma lucidum* enhances its antioxidant activities in vitro. *Carbohydr. Polym.* **2009**, *78*, 227−234.

(5) Lin, K.; Kao, Y.; Kuo, H. K.; Yang, W. B.; Chou, A.; Lin, H. H.; Yu, A.; Wong, C. H. Reishi polysaccharides induce immunoglobulin production through the TLR4/TLR2-mediated induction of transcription factor blimp-1. *J. Biol. Chem.* **2006**, *281*, 24111−24123.

(6) Shao, B. M.; Dai, H.; Xu, W.; Lin, Z. B.; Gao, X. M. Immune receptors for polysaccharides from *Ganoderma lucidum*. *Biochem. Biophys. Res. Commun.* **2004**, *323*, 133−141.
(7) Yuen, J. W. M.; Gohel, M. D. I. The dual roles of ganoderma antioxidants on urothelial cell DNA under carcinogenic attack. *J. Ethnopharmacol.* **2008**, *118*, 324−330.
(8) Zhang, M.; Cui, S. W.; Cheung, P. C. K.; Wang, Q. Antitumor polysaccharides from mushrooms: a review on their isolation process, structural characteristics and antitumor activity. *Trends Food Sci. Technol.* **2007**, *18*, 4−19.
(9) Zhao, L. Y.; Dong, Y. H.; Chen, G. T.; Hu, Q. H. Extraction, purification, characterization and antitumor activity of polysaccharides, from *Ganoderma lucidum*. *Carbohydr. Polym.* **2010**, *80*, 783−789.
(10) Chen, T. Q.; Wu, J. Z.; Zhu, J. R. GC-MS analysis of chemical components in the essential oils from the ultrafine-powder of *Ganoderma sinense*. *Mycosystema* **2007**, *26*, 279−283.
(11) Liu, C.; Zhao., F.; Chen, R. Y. A novel alkaloid from the fruiting bodies of *Ganoderma sinense*. *Chin. Chem. Lett.* **2010**, *21*, 197−199.
(12) Han, F.; Liu, Y.; Guo, L. Q.; Zeng, X. L.; Liu, Z. M.; Lin, J. F. Heterologous expression of the immunomodulatory protein gene from *Ganoderma sinense* in the basidiomycete *Coprinopsis cinerea*. *J. Appl. Microbiol.* **2010**, *109*, 1838−1844.
(13) Li, Q. Z.; Wang, X. F.; Huang, L.; Zhou, W. X. Fungal immunomodulatory protein from *Ganoderma sinense* expressed in *E. coli* and the identification of immunocopetence. *Act. Bot. Bor. Occ. Sinica* **2010**, *30*, 35−40.
(14) Liu, Y. W.; Gao, J. L.; Guan, J.; Qian, Z. M.; Feng, K.; Li, S. P. Evaluation of antiproliferative activities and action mechanisms of extracts from two species of *Ganoderma* on tumor cell lines. *J. Agric. Food Chem.* **2009**, *57*, 3087−3093.
(15) Liu, C.; Wang, H. Q.; Li, B. M.; Chen, R. Y. Studies on chemical constituents from the fruiting bodies of *Ganoderma sinense*. *Chin. J. Chin. Mater. Med.* **2007**, *32*, 235−237.
(16) Qiao, Y.; Zhang, X. M.; Qiu, M. H. Two novel triterpenoids from *Ganoderma sinense*. *Molecules.* **2007**, *12*, 2038−2046.
(17) Qian, J. Y.; Chen, W.; Zhang, W. M.; Zhang, H. Adulteration identification of some fungal polysaccharides with SEM, XRD, IR and optical rotation: a primary approach. *Carbohydr. Polym.* **2009**, *78*, 620−625.
(18) Sato, N.; Ma, C. M.; Komatsu, K.; Hattori, M. Triterpene-farnesyl hydroquinone conjugates from *Ganoderma sinense*. *J. Nat. Prod.* **2009**, *72*, 958−961.
(19) Sato, N.; Zhang, Q.; Ma, C. M.; Hattori, M. Anti-human immunodeficiency virus-1 protease activity of new lanostane-type triterpenoids from *Ganoderma sinense*. *Chem. Pharm. Bull.* **2009**, *57*, 1076−1080.
(20) Lawrence, J. F.; Lyengar, J. R. Gas chromatographic determination of polysaccharide gums in foods after hydrolysis and derivatization. *J. Chromatogr.* **1985**, *350*, 237−244.
(21) Needs, P. W.; Selvendran, R. R. Avoiding oxidative degradation during sodium hdroxide/methyl iodide-mediated carbohydrate methylation in dimethyl sulfoxide. *Carbohydr. Res.* **1993**, *245*, 1−10.
(22) Sweet, D. P.; Shapiro, R. H.; Albersheim, P. Quantitative analysis by various g.l.c. response factor theories for partially methylated and partially ethylated alditol acetates. *Carbohydr. Res.* **1995**, *40*, 217−225.
(23) Yue, G. G.; Chan, B. C.; Hon, P. M.; Kennelly, E. J.; Yeung, S. K.; Cassileth, B. R.; Fung, K. P.; Leung, P. C.; Lau, C. B. Immunostimulatory activities of polysaccharide extract isolated from *Curcuma longa*. *J. Biol. Macromol.* **2010**, *47*, 342−347.
(24) Chan, B. C.; Yu, H.; Wong, C. W.; Lui, S. L.; Jolivalt, C.; Ganem-Elbaz, C.; Paris, J. M.; Morleo, B.; Litaudon, M.; Lau, C. B.; Ip, M.; Fung, K. P.; Leung, P. C.; Han, Q. B. Quick identification of kuraridin, a noncytotoxic anti-MRSA (methicillin-resistant *Staphylococcus aureus*) agent from *Sophora flavescens* using high-speed counter-current chromatography. *J Chromatogr B.* **2012**, *880*, 157−162.
(25) Cao, L. Z.; Lin, Z. B. Regulation on maturation and function of dendritic cells by *Ganoderma lucidum* polysaccharides. *Immunol. Lett.* **2002**, *83*, 163−169.
(26) Staub, A. M. Removal of protein-Sevag method. *Methods Carbohydr. Chem.* **1965**, *5*, 5−6.
(27) Jiménez, E.; Garcia-Lora, A.; Martinez, M.; Garrido, F. Identification of the protein components of protein-bound polysaccharide (PSK) that interact with NKL cells. *Cancer Immunol. Immunother.* **2005**, *54*, 395−399.
(28) Ng, T. B. A review of research on the protein-bound polysaccharide (polysaccharopeptide, PSP) from the mushroom *Coriolus versicolor* (Basidiomycetes: Polyporaceae). *Gen. Pharm.* **1998**, *30*, 1−4.
(29) Ojha, A. K.; Mondal, D.; Chandra, K.; Mondal, S.; Das, D.; Roy, S. K.; Ghosh, K.; Islam, S. S. Structural assignment of a heteropolysaccharide isolated from the gum of *Cochlospermum religiosum* (Katira gum). *Carbohydr. Res.* **2008**, *343*, 1222−1231.
(30) Roy, S. K.; Das, D.; Mondal, S.; Maiti, D.; Bhunia, B.; Maiti, T.; Islam, S. S. Structural studies of an immunoenhancing water-soluble glucan isolated from hot water extract of an edible mushroom, *Pleurotus florida*, cultivar Assam Florida. *Carbohydr. Res.* **2009**, *18*, 2596−2601.
(31) Peng, Y. F.; Zhang, L. N.; Zeng, F. B.; Kennedy, J. F. Structure and antitumor activities of the water-soluble polysaccharides from *Ganoderma tsugae* mycelium. *Carbohydr. Polym.* **2005**, *59*, 385−392.
(32) Leung, M. Y. K.; Liu, C.; Koon, J. C. M.; Fung, K. P. Polysaccharide biological response modifiers. *Immunol. Lett.* **2006**, *105*, 101−114.
(33) Chen, J.; Seviour, R. Medicinal important of fungal β-(1→3), (1→6) glucans. *Mycol. Res.* **2007**, *111*, 635−652.
(34) Samarasinghe, R.; Tailor, P.; Tamura, T.; Kaisho, T.; Akira, S.; Ozato, K. Induction of an anti-inflammatory cytokine, IL-10, in dendritic cells after toll-like receptor signaling. *J. Interferon Cytokine Res.* **2006**, *26*, 893−900.