JPP Journal of Pharmacy and Pharmacology
ROYAL PHARMACEUTICAL SOCIETY

JPP Journal of Pharmacy and Pharmacology

JPP 2011, 63: 825–832

Received April 27, 2010
Accepted February 21, 2011
DOI
10.1111/j.2042-7158.2011.01274.x
ISSN 0022-3573

Research Paper

Isolation of a polysaccharide with antiproliferative, hypoglycemic, antioxidant and HIV-1 reverse transcriptase inhibitory activities from the fruiting bodies of the abalone mushroom *Pleurotus abalonus*

Chang Rong Wang[a], Tzi Bun Ng[b], Le Li[c], Jin Cen Fang[c], Yun Jiang[a], Ting Yi Wen[d], Wen Tao Qiao[a], Ning Li[a] and Fang Liu[a]

[a]Center for AIDS Research, Department of Microbiology, Nankai University, Tianjin, [b]School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong, [c]Research Center of Fishery Production Standard & Quality, Chinese Academy of Fishery Sciences and [d]Key Laboratory of Systems Microbial Biotechnology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China

Abstract

Objective The intent of this study was to purify and characterize a polysaccharide named LA from the fruiting bodies of the edible mushroom Pleurotus abalones.

Methods The 120-kDa polysaccharide was obtained by extraction with boiling water, ethanol precipitation, ion exchange chromatography on Diethylaminoethyl-cellulose and gel filtration on Sephadex G-200.

Key findings The LA was composed of glucose, rhamnose, glucuronic acid, xylose, galactose and arabinose in the molar ratio of 26.3 : 2.7 : 1 : 1.4 : 1.8 : 1.2. The FITR and ^{1}H-NMR spectrum of LA disclosed that it was a saccharide with an α-configuration. Its ^{13}C-NMR spectrum revealed that its main chain was $[\rightarrow 6)$-α-D-Gly$(1\rightarrow]_n$. The LA exhibited antioxidant activities, especially in scavenging 1,1- diphenyl-2-picryl-hydrazyl radicals and hydroxyl radicals. It manifested antiproliferative activity towards breast cancer MCF7 cells with an IC50 of 3.7 μM, and also exerted some antiproliferative activity against HepG2 cells. It manifested a hypoglycemic action on diabetic mice. It inhibited HIV-1 reverse transcriptase with an IC50 of 8.7×10^{-2} μM.

Conclusions The polysaccharide from the abalone mushroom.

Keywords antioxidant; antiproliferative; *Pleurotus abalonus*; polysaccharide

Introduction

Edible mushrooms are highly nutritious. They exhibit tonic and medicinal attributes and are thus used in folk medicine.[1] The nutritional values and taste components of some mushrooms have been studied.[2] Much attention has been paid to their medicinal value, such as their antioxidative, antitumor, immunomodulating, antiviral, hypocholesterolemic and hepatoprotective activities.[3–7]

The biologically active compounds that have been reported from mushrooms encompass polysaccharides, terpenoids, polysaccharide-peptide complexes and proteins.[4,8] An extract of the winter mushroom exhibited antioxidant activity.[9] The biological activities of *Pleurotus ostreatus*, including its antioxidant activity, have been extensively investigated.[10] Its aqueous extract exerted antiproliferative and proapoptotic effects on HT-29 colon cancer cells.[11] Ethanolic extracts from *P. citrinopileatus* fruiting bodies and mycelia, and the filtrate from its submerged culture demonstrated antioxidant activity.[12]

Various species of *Pleurotus* have been shown to possess a diversity of medicinal properties, such as antitumour,[13] immunomodulatory,[14] antioxidant,[15] anti-inflammatory,[16] antihyperglycemic,[12] antimicrobial[17] and antiviral activities.[18] Compounds isolated from *Pleurotus* spp., including fermentation broth extracts of its mycelia and fruit bodies, exhibited these therapeutic activities. In particular, polysaccharides appeared to display potent antitumour and immuno-enhancing activities. However, the biochemical mechanisms of these therapeutic activities still remain largely unknown.

Correspondence: Tzi Bun Ng, School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong, China.
E-mail: biochemistry@cuhk.edu.hk

Pleorotus abalonus belongs to class Basidiomycetes, order Agaricales and family Pleurotaceae. A previous study showed that the methanolic extract of *Pleurotus abalonus* was more potent in antioxidant activity, reducing power and free-radical scavenging ability, and had a higher total phenol content than those of winter and shiitake mushrooms.[9] By comparison, there is a dearth of information on *Pleurotus abalones*. No polysaccharide has been identified in the fruiting body of *Pleurotus abalonus*. We have screened the extracts of 37 edible fungi for antioxidant activity *in vitro* (data not shown) and found that the extract from *Pleurotus abalonus* manifests antioxidant activity.

Polysaccharides, which are widely distributed in animals, plants and microorganisms, have been shown to play an important role as dietary free-radical scavengers with a protective action against oxidative damage. Accumulating evidence indicates that reactive oxygen species produced by sunlight, ultraviolet light, ionizing radiation, chemical reactions or metabolic processes exert a wide range of pathological effects, such as eliciting DNA damage, carcinogenesis and cellular degeneration related to ageing.[19–21] Polysaccharides play important roles as thickening, stabilizing and gelling agents in many foods. Moreover, in emulsion systems polysaccharides are very often employed to improve emulsion stability and textural properties. Polysaccharides modify and control the rheological properties of aqueous systems, film formers, lubricants and friction reducers.[22]

The aim of this study was to ascertain an efficient method for extraction, isolation and purification of the polysaccharide from the fruiting bodies of *Pleurotus abalonus* as well as to analyse its structure, which would allow an insight into how this affected its bioactivity. Chemical properties, FTIR and NMR spectra were used for chemical characterization.

Materials and Methods

Materials

Dried abalone mushroom (*Pleurotus abalonus*), which was cultured and then collected in Henan Luoyang on September 2007, was purchased from the Henan University of Science and Technology by Dr Jun Hou and authenticated by him. A sample of the mushroom was deposited in the Department of Microbiology, Nankai University, with the accession number LF-PA92007. Sephadex G-200 was from GE Healthcare (Hong Kong, China). Diethylaminoethyl-cellulose 32, cytochrome C, thiourea, 2,2-azo-bis-(2-amidinopropane) dihydrochloride, dextran-blue 2000 and 1,1-diphenyl-2-picryl-hydrazyl (DPPH), were purchased from Sigma Aldrich (St Louis, MO, USA). All other chemicals used were of reagent grade.

Isolation and purification of the samples

Dried fruiting bodies of the mushroom were washed, dried and cut into pieces before immersion in distilled water overnight at room temperature. They were then boiled in distilled water for 3 h. After filtration to remove debris, the filtrate was concentrated in a rotary evaporator. Four volumes of ethanol were added, and then the mixture was allowed to stand at room temperature overnight before filtration in order to collect the precipitate. This was lyophilized to yield the crude polysaccharide sample, which we named P.

P was dissolved in 10 mM tris-HCl buffer (pH 7.4) and subjected to ion exchange chromatography on a 2.5×50 cm column of DEAE-cellulose, to yield an unadsorbed fraction named T. The adsorbed fraction, designated L, was eluted with 1 M NaCl and then chromatographed on a 2.0×100 column of Sephadex G-200 in distilled water to yield the polysaccharide LA.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and periodic acid schiff (PAS) analysis

The method of SDS-PAGE as described by Lyon *et al*. was used.[23]

Polysaccharide contents and monosaccharide analysis

The carbohydrate content of LA was determined with the anthrone-sulphuric acid colorimetric method using glucose as standard.[24] LA (20 mg) was hydrolysed with 2.0 ml 1 M sulphuric acid at 100°C for 6 h. Then 2 M NaOH was added to adjust the pH to 7.0 and distilled water added to dilute the mixture to 5.0 ml. The mixture was centrifuged, and 80 µl of the supernatant was collected, then 80 µl 0.25 M 1-(2-chlorophenyl)-3-methyl-2-pyrazolin-5-one (dissolved in methanol) and 80 µl 0.2 M NaOH were added, followed by incubation at 70°C for 30 min. The pH was adjusted to 7.0 and the mixture was extracted with 0.5 ml isoamyl acetate. The organic phase was discarded. Next 0.5 ml chloroform was added for re-extraction. The aqueous phase was chromatographed on a C18 column (250 mm × 4.6 mm, 5 µm). Phase A was 15% (v/v) acetonitrile and 20 mM ammonium acetate, and phase B was 40% (v/v) acetonitrile and 20 mM ammonium acetate. From 0 to 25 min, phase B was changed from 0 to 50% (v/v). The wavelength for monitoring the column effluent was 250 nm and the flow rate was 1 ml/min.

Protein content

The protein concentration of LA was determined using the method of Lowry *et al*.,[25] with bovine serum albumin as standard.

Structural characterization by FTIR, ^{13}C NMR and ^{1}H NMR spectroscopy

LA was analysed by transmittance IR spectroscopy in the form of KBr disks using a Perkin Elmer Spectrum 1000 FTIR spectrometer. ^{1}H and ^{13}C NMR spectra of solutions in D_2O were recorded at ambient temperature using a Varian Associates Unity 500 spectrometer, which was operated at 500 MHz for ^{1}H and 125.8 MHz for ^{13}C.

Assay of inhibition of erythrocyte hemolysis

Blood cells were obtained as described by Miki *et al*.[26] Erythrocyte hemolysis was mediated by peroxyl radicals in the assay system.[27] The percentage of hemolysis was calculated by the equation $(1 - OD_{PBS}/OD_{H2O}) \times 100\%$. L-ascorbic acid was used as a positive control.

Assay of free-radical scavenging activities

The ability of LA to scavenging DPPH radicals and hydroxyl radicals was tested.[28] L-ascorbic acid was used as a positive control in the former assay and thiourea in the latter.

Assay of antiproliferative activity on MCF7 cells and HepG2 cells

Mammary cancer (MCF7) cells and hepatoma (HepG2) cells were separately suspended in Roswell Park Memorial Institute medium and adjusted to 2×10^4 cells/ml. A sample of 100 µl of the cell suspension was seeded to a well of a 96-well plate, followed by incubation for 24 h. Different concentrations of LA in 100 µl complete Roswell Park Memorial Institute medium were then added to the wells and incubated for 24 or 48 h. After that, 20 µl of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) at a concentration of 5 mg/ml in phosphate-buffered saline was spiked into each well and the plates were incubated for 4 h. The plates were then centrifuged at 324 × g for 5 min. The supernatant was carefully removed and 150 µl of dimethyl sulfoxide was added to each well to dissolve the MTT-formazan at the bottom of the wells. After 10 min, the absorbance was measured at 590 nm by microplate reader.[29]

Assay for HIV reverse transcriptase inhibitory activity

The ability of LA to inhibit HIV-1 reverse transcriptase was assessed using an ELISA kit from Boehringer Mannheim (Germany) as described by Collins *et al*.[30] Azidothymidine was employed as a positive control.

Assay of hypoglycemic activity

Approval for conducting these experiments was obtained from the Animal Ethics Committee of Nankai University. Normal male Kunming mice were randomly divided into three groups of 15 mice each: a CT (diabetic control) group, a BHA group (treated with butylated hydroxyanisole) and an LA group (treated with LA), respectively. The mice in the LA, BHA and CT groups were treated with LA (1 mg/kg), BHA (1 mg/kg) and 0.9% NaCl orally for 10 days. In this assay, BHA was used as the positive control. On the tenth day, all mice received tetraoxypyrimidine (90 mg/kg) by injection to induce hyperglycemia. Their fasting blood glucose levels were tested on the 12th, 16th and 20th days by taking blood from the caudal vein.

Statistical analysis

The mean value and standard deviation (SD) were calculated and expressed. Some results were analysed by Students' *t*-test. One-way analysis of variance (ANOVA) was applied to the results followed by the Tukey test. $P < 0.05$ was considered to represent a significant difference. GraphPad Prism 5 Demo software was used to make comparisons between two groups.

Results

Identification of LA

SDS-PAGE and staining with Coomassie Brilliant Blue and PAS

In SDS-PAGE, purified LA appeared as a single band. The protein marker in lane 1, which was used as a negative control, could not be stained by PAS. The LA band in lane2 did not stain with Coomassie Brilliant Blue but stained red with PAS, which indicated that it was a polysaccharide (Figure 1). The molecular mass of LA was determined by gel filtration on Sephadex G-200 with the following standards: dextran blue 2000, bovine serum albumin (67 kDa), ovalbumin (43 kDa) and cytochrome C (12.5 kDa). LA had a molecular mass of 120 kDa.

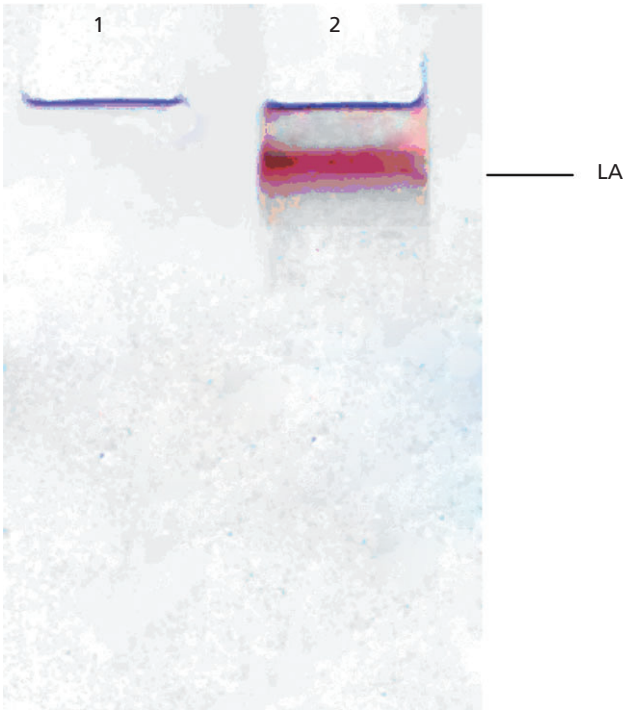

Figure 1 SDS-PAGE and PAS staining of LA. Lane 1, protein marker (negative control, which was not stained due to absence of carbohydrate); lane2, sample LA (stained red by PAS indicating presence of polysaccharide).

Table 1 Yields and carbohydrate and protein contents of different fractions

Fraction	Carbohydrate (%)	Protein (%)
P	37.2 ± 1.1	3.8 ± 0.2
L	28.2 ± 1.0	6.1 ± 0.9
LA	92.6 ± 2.8	0.1 ± 0.06

The values are mean ± SD ($n = 3$).

Carbohydrate and protein analyses

The carbohydrate and protein contents of all the samples are shown in Table 1. The carbohydrate content of LA was 92.6% and its protein content was negligible, showing that LA is a pure polysaccharide.

Results of HPLC analysis revealed that LA contains glucose, rhamnose, glucuronic acid, xylose, galactose and arabinose. The molar ratio of these monosaccharides was 26.3 : 2.7 : 1 : 1.4 : 1.8 : 1.2 (Figure 2). Glucose was the major

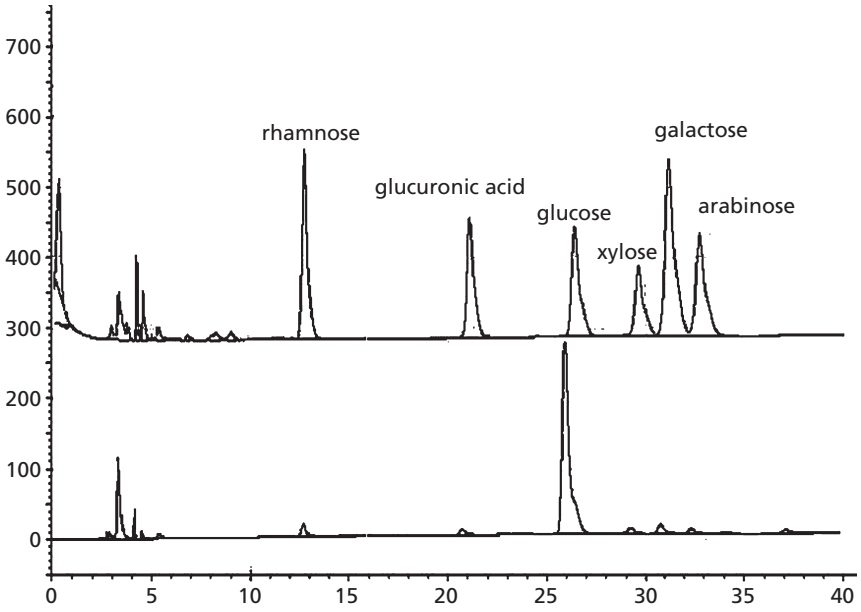

Figure 2 Carbohydrate analysis of LA and standard carbohydrates. Standards in panel above, LA in panel below.

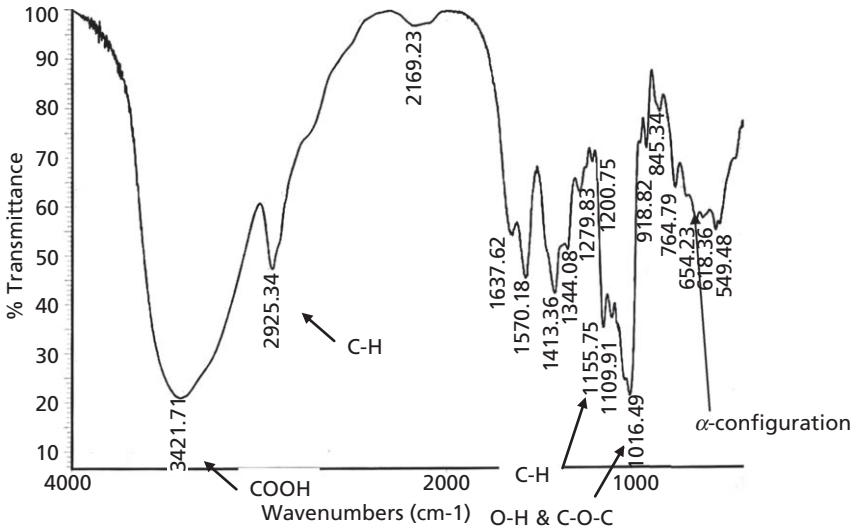

Figure 3 FTIR spectrum of LA.

monosaccharide in LA. Many bioactive polyssacharides extracted from fungi are comprised mainly of glucose.

Structural characterization of LA by FTIR, ^{13}C NMR and ^{1}H NMR spectroscopy

The FT-IR spectrum of purified polysaccharide LA measured in KBr pellets is shown in Figure 3. The peak of 3421.71 cm^{-1} in the range of 3500–3100 cm^{-1} was due to ν (O–H) and ν (N–H) stretching and included the hydrogen bonds in and between molecules. The wide peak in the range of 3600–3000 cm^{-1} was attributed to COOH stretching. The peak at 2925.34 cm^{-1} in the range of 3000–2800 cm^{-1} was ascribed to C–H stretching in polysaccharides. The peaks in the range of 1400–1200 cm^{-1} represented the variable-angle vibrations of C–H in polysaccharides. The data above show that LA is a polysaccharide. The peak at 1108 cm^{-1} was due to the vibrations of –OH and C–O–C in the pyranoid ring. The peak at 845 cm^{-1} indicated that LA is a polysaccharide with an α configuration.

The ^{1}H NMR spectrum of the purified polysaccharide LA is shown in Figure 4. All the signals of LA were in the range δ3.5–5.5 ppm. Generally, the vibrations at δ4.8–5.3 ppm indicate that the polysaccharide has an α-configuration, while the vibrations of a β-configuration were at 4.0–4.8 ppm. The signals of LA were around δ5.0 ppm, which shows that it is a polysaccharide with both α and β configurations. The main signals were at δ4.8–5.3 ppm, indicating that the main chain had an α-configuration.

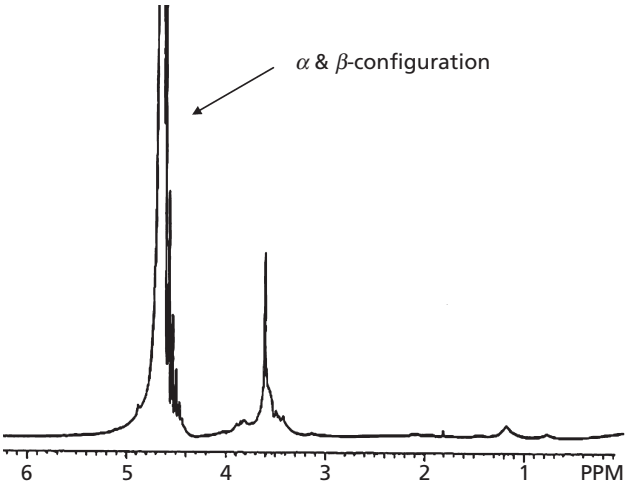

Figure 4 The ^{1}H NMR spectrum of LA.

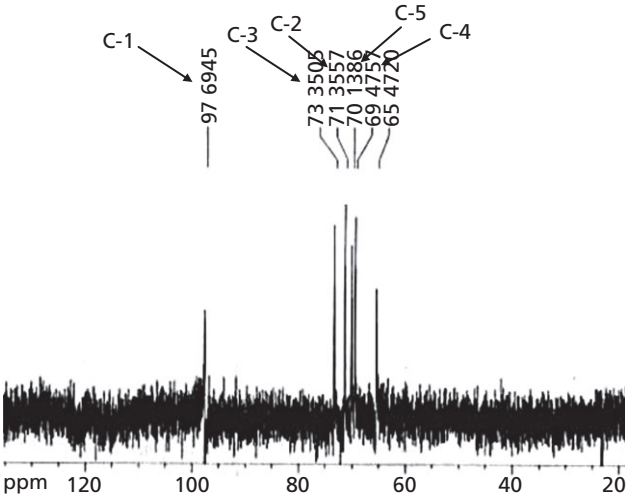

Figure 5 The ^{13}C NMR spectrum of LA.

The ^{13}C NMR spectrum of the purified polysaccharide LA is shown in Figure 5. The peaks at 97.69, 71.36, 73.35, 69.48 and 70.14 ppm were the signals of C-1, C-2, C-3, C-4 and C-5, respectively. The ^{13}C NMR spectrum shows that the main chain of LA is [→6)-α-D-Glc*p*-(1→]n.

Antioxidant activities of fractions

The crude sample P showed antioxidant activity in an assay of hemolysis inhibition. The two fractions T and L, derived from P, had very different inhibition rates, with L showing much higher activity than T. LA extracted from L showed antioxidant activities in the assay of hemolysis (Table 2).

Assay of free-radical scavenging activities

LA showed antioxidant activity, especially in scavenging DPPH radicals and hydroxyl radicals (Table 3). The IC50 of LA was 90.2 μg/ml (i.e. 0.75 μM) in the assay of DPPH radical scavenging and 111.7 μg/ml (i.e. 0.93 μM) in the hydroxyl radical scavenging.

Table 2 Effects of various fractions on inhibition of erythrocyte hemolysis

Fraction	Concentration (μg/ml)	Inhibition of hemolysis (%)
P	500	79.8 ± 2.2^{d}
T	500	22.0 ± 3.3^{b}
L	500	86.7 ± 2.0^{e}
LA	500	62.9 ± 0.1^{c}
Positive control*	500	92.0 ± 1.0^{e}
Negative control**	–	8.4 ± 1.2^{a}

*Positive control was L-ascorbic acid. **Negative control was phosphate buffered saline. The values are mean ± SD ($n = 3$) Different letters indicate statistically significant differences between activities of different samples by students' *t*-test ($P < 0.05$), while the same letter indicates no significant difference.

Table 3 Effects of LA on scavenging of DPPH radicals and hydroxyl radicals

Concentration (μM)	Inhibition of DPPH radicals (%)	Inhibition of hydroxyl radicals (%)
1.67	77.4 ± 1.6^{c}	71.1 ± 2.3^{c}
0.835	58.8 ± 2.1^{b}	40.7 ± 1.5^{b}
0.418	25.5 ± 1.4^{a}	27.8 ± 1.6^{a}

The values are mean ± SD ($n = 6$). Different letters indicate statistically significant differences between activities of LA at different concentrations by students' *t*-test ($P < 0.05$), while the same letter indicates no significant difference. Thiourea at a concentration of 100 ug/ml produced 99.21 ± 2.6%.

Table 4 Inhibitory effects of LA on MCF7 and HepG2 cells

Concentration (μM)	Inhibition of MCF7 cells (%)	Inhibition of HepG2 cells (%)
5	65.2 ± 3.1^{c}	34.7 ± 1.8^{c}
2.5	20.9 ± 1.3^{b}	14.2 ± 0.9^{b}
1.25	15.3 ± 1.1^{a}	12.5 ± 1.1^{b}
0.625	14.5 ± 0.7^{a}	8.0 ± 1.3^{a}

The values are mean ± SD ($n = 6$). Data within each column bearing different letters exhibit statistically significant differences between activities of LA at different concentrations by students' *t*-test ($P < 0.05$), while those with the same letter have no significant difference.

Antiproliferative activity on MCF7 cells and HepG2 cells

The IC50 of LA in the assay of antiproliferative activity on MCF7 cells was 446.7 μg/ml (i.e. 3.7 μM). LA also had some antiproliferative activity on HepG2 cells (Table 4).

Table 5 Inhibitory effect of LA on HIV-1 reverse transcriptase

Concentration (μM)	Inhibition of HIV-1 reverse transcriptase (%)
2	60.5 ± 3.2[d]
1	57.2 ± 2.7[c]
0.5	54.2 ± 3.6[b]
0.25	52.4 ± 1.9[b]
0.1	50.7 ± 4.1[a]

The values are mean ± SD ($n = 6$). Different letters indicate statistically significant differences between activities of LA at different concentrations by students' t-test ($P < 0.05$), while the same letter indicates no significant difference. Azidothymidine at a concentration of 2 μM produced 92.6 ± 1.3% inhibition.

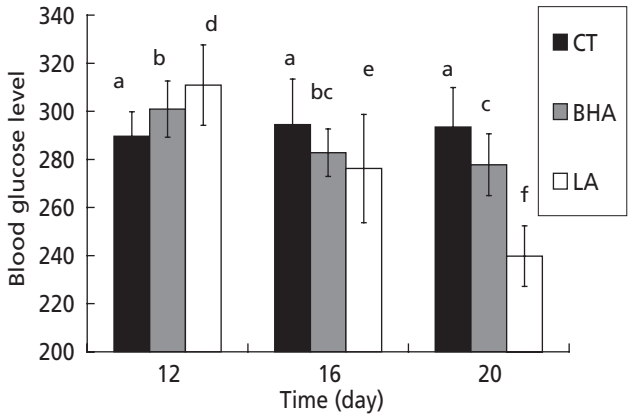

Figure 6 Effect of LA on blood glucose level in diabetic mice after different durations of treatment (mean ± SD, $n = 6$). One-way-analysis of variance (ANOVA) was used to analyse the results followed by the Tukey test. Different letters indicate statistically significant difference in blood glucose level after treatment for different durations ($P < 0.05$). (a) No statistically significant difference after different durations of treatment.for the data of group CT. (b and c) Statistically significant difference after different durations of treatment for the data of group BHA (1 mg/kg). (d, e and f) Statistically significant difference after different durations of treatment.for the data of group LA (1 mg/kg).

HIV-1 reverse transcriptase inhibitory activity

LA inhibited HIV-1 reverse transcriptase, with an IC50 value of 10.4 μg/ml (i.e. 87 nM) (Table 5).

Hypoglycemic activity

LA reduced the blood glucose level of drug-induced diabetic mice, with a higher activity than the positive control, BHA (Figure 6). BHA and LA were given by daily oral administration at 1 mg/kg starting on day 1. Tetraoxypyrimidine was given on day 10 to induce hyperglycemia. The blood glucose level of the mice treated with LA decreased by 22.9% while in those treated with BHA it only decreased by 8.3%. The blood glucose level of the mice in CT group did not show any decrease.

Discussion

In a recent study, many active polysaccharides with new structures of the main chain have been found. It is known that β-D-glucans, but not α-D-glucans, have strong antitumor activities. Consequently, it is natural that the crude polysaccharide named LA would be similar to β-D-glucan, which had effect on the immune responses.[31] There are many reports showing that most polysaccharides with the main chain configuration of α-(1→6)-D-glucan do not have biological activity.[32] Polysaccharides represent a structurally diverse class of biological macromolecules with a wide-range of physicochemical properties. Many mushroom polysaccharides and polysaccharide–protein complexes have been considered as possible antitumor and immunomodulating agents. There have been many reports on polysaccharides extracted from *Ganoderma lucidum*. The major bioactive *Ganoderma* polysaccharide species are β-1-3 and β-1-6-D-glucans. The structure is β-1-3-D-glucopyranan with 1–15 units of β-1-6 monoglucosyl side chains. These showed many biological activities, such as antitumor and anti-HSV.[33]

In this study, a novel polysaccharide LA with α-glycosidic bond was extracted from the fruit bodies of *Pleurotus abalonus* for the first time. LA with the configuration [→6)-α-D-Glc*p*-(1→]n glucopyranose showed antioxidant activity, free-radical scavenging activity, antiproliferative activity on MCF7 cells and HepG2 cells, anti-HIV-1 reverse transcriptase activity and hypoglycemic activity. All these results indicate that some α-D-glycan also show antitumor activity and that the polysaccharide with the main chain [→6)-α-D-Glc*p*-(1→]n glucopyranose could have many biological activities.

Antioxidant activity is an important characteristic of many biological macromolecules. Superoxide and hydroxyl radicals are the two most representative free radicals. In cellular oxidation reactions, the formation of a superoxide radical normally comes first. Moreover, the effects of superoxide radicals can be magnified by their producing other kinds of cell-damaging free radicals and oxidizing agents. It is hydroxyl radicals that have the most damaging action among free radicals.[34] Inhibition of hemolysis is a common and quick method to test the antioxidant activities of samples. Antioxidants protect the erythrocyte, which can be affected by many effectors. Thus, in this study, the hemolysis inhibition test was used as an initial step for screening the active fractions separated from the fruit body extract of *Pleurotus abalonus*.

Scavenging assays of DPPH and hydroxyl radicals were used to test the antioxidant ability of the samples directly. A polysaccharide with a 1013 kDa molecular weight, purified from the *Ganoderma atrum*, showed antioxidant activity in the DPPH-scavenging assay.[35] Its inhibition rate in that test was 76.9% at a concentration of 1 mg/ml, while that of LA was lower than 0.2 mg/ml at the same inhibition rate. LA showed some antioxidant activity in an assay of erythrocyte hemolysis and high activities in scavenging DPPH and hydroxyl radicals, which indicates that it is an effective agent for scavenging free radicals but not for preventing the generation of free radicals.

Some researchers have shown that mushroom polysaccharide complexes such as Polysaccharopeptide Krestin, a protein-bound polysaccharide extracted from the mycelia of *Coriolus versicolor*, and the polysaccharide–protein complex (PSPC), isolated from the culture filtrate of *Tricholoma lobayense*, also have antitumor activities. However, the antitumor activities of pure polysaccharides are seldom reported.[36,37]

In an assay of antiproliferative activity on MCF7 cells and HepG2 cells, LA showed stronger inhibition on MCF7 cells than HepG2 cells. The inhibition ratio and the concentration of the sample showed a linear relationship. Moreover, the antiproliferative activity on MCF7 of LA (IC50 = 3.7 μM) was also stronger than that of lentinan from *Lentinus edodes* (IC50 = 6.9 μM), which has been noted for its antitumor and immunomodulating activity. This indicates that LA has potent antitumor activity *in vitro*.

A variety of macromolecule compounds with inhibition activity on HIV-1 reverse transcriptase have been found in recent years. However, pure polysaccharides with this activity are seldom reported. The polysaccharopeptide from *Coriolus versicolor* potently inhibited recombinant HIV-1 reverse transcriptase (IC50 = 6.2 μg/ml).[38] LA also shows inhibition activity on HIV-1 reverse transcriptase, with IC50 = 10.4 μg/ml. This indicates that LA could be developed into a potent inhibitor of HIV-1 reverse transcriptase.

In the test of hypoglycemic activity, LA showed stronger activity than that of the positive control, BHA. The activity appeared 2–4 days after injection of the diabetes-inducing agent tetraoxypyrimidine, and the hypoglycemic effect persisted for more than 10 days. This shows that LA could decrease the blood glucose level of diabetes mice quickly, efficiently and persistently.

Most of the antitumour glucans from *Ganoderma lucidum*, with an average molecular weight of 1050 kDa, were reported to be insoluble and contain a branched glucan core.[39] But the polysaccharide extracted from *Pleurotus abalones* was only 120 kDa. It is a small polysaccharide with many biological activities, so it may have considerable value in medicine.

Conclusion

LA is a novel polyssacharide that was first isolated and purified from the fruit bodies of the edible mushroom *Pleurotus abalones* in the present study. Its molecular weight is 120 kDa. Its main chain of polysaccharide is [→6)-α-D-Glc*p*-(1→]n glucopyranose. LA shows high antioxidant activity, especially in scavenging free radicals, with an IC50 of 0.75 μM for DPPH radical scavenging and 0.93 μM for hydroxyl radical scavenging. It may inhibit proliferation of t MCF7 and HepG2 tumour cells. Moreover, LA shows inhibitory activity on HIV-1 reverse transcriptase, with an IC50 of 8.7×10^{-2} μM and also decreases the blood glucose level of drug-induced diabetic mice by 22.9%. Thus, LA displays a variety of biological activities that could have potential medicinal value.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Acknowledgements

We thank support of National Natural Science Foundation (grant no. 30970021), Tianjin Natural Science Foundation (grant no.10JCYBJC09800), National Key Scientific Program of China ((grant no. 2008ZX09401-05), Chinese Ministry of Health (grant no. 2008ZX10001-002) and Miss Fion Yung for excellent secretarial assistance.

References

1. Sheu F *et al*. Isolation and characterization of an immunomodulatory protein (APP) from the Jew's Ear mushroom *Auricularia polytricha*. *Food Chem* 2004; 87: 593–600.
2. Yang JH *et al*. Non-volatile taste components of several commercial mushrooms. *Food Chem* 2001; 72: 465–471.
3. Breene WM. Nutritional and medicinal value of specialty mushrooms. *J Food Prot* 1990; 53: 883–894.
4. Wasser SP, Weis AL. Therapeutic effects of substances occurring in higher Basidiomycetes mushrooms: a modern perspective. *Crit Rev Immunol* 1999; 19: 65–96.
5. Salah-Abbès JB *et al*. In-vitro free radical scavenging, antiproliferative and anti-zearalenone cytotoxic effects of 4-(methylthio)-3-butenyl isothiocyanate from Tunisian Raphanus sativus. *J Pharm Pharmacol* 2010; 62: 231–239.
6. Li QQ *et al*. Antineoplastic effect of beta-elemene on prostate cancer cells and other types of solid tumour cells. *J Pharm Pharmacol* 2010; 62: 1018–1027.
7. Selegean M *et al*. Effect of the polysaccharide extract from the edible mushroom *Pleurotus ostreatus* against infectious bursal disease virus. *Int J Mol Sci* 2009; 10: 3616–3634.
8. Wang HX *et al*. Lectins from mushrooms. *Microbiol Res* 1998; 102: 897–906.
9. Bor JY *et al*. Evaluation of antioxidant activity and inhibitory effect on nitric oxide production of some common vegetables. *J Agric Food Chem* 2006; 54: 1680–1686.
10. Bobek P *et al*. Antioxidative effect of oyster mushroom (*Pleurotus ostreatus*) in hypercholesterolemic rat. *Pharmazie* 1995; 50: 441–442.
11. Lavi I *et al*. An aqueous polysaccharide extract from the edible mushroom *Pleurotus ostreatus* induces anti-proliferative and pro-apoptotic effects on HT-29 colon cancer cells. *Cancer Lett* 2006; 244: 61–70.
12. Hu SH *et al*. Antihyperlipidemic and antioxidant effects of extracts from *Pleurotus citrinopileatus*. *J Agric Food Chem* 2006; 54: 2103–2110.
13. Gu YH, Sivam G. Cytotoxic effect of oyster mushroom *Pleurotus ostreatus* on human androgen-independent prostate cancer PC-3 cells. *J Med Food* 2006; 9: 196–204.
14. Wang JC *et al*. Optimization for the production of water-soluble polysaccharide from *Pleurotus citrinopileatus* in submerged culture and its antitumor effect. *Appl Microbiol Biotechnol* 2005; 67: 759–766.
15. Yang JH *et al*. Antioxidant properties of several commercial mushrooms. *Food Chem* 2002; 77: 229–235.
16. Bobek P *et al*. Effect of pleuran (β-glucan from *Pleurotus ostreatus*) in diet or drinking fluid on colitis in rats. *Nahrung* 2001; 45: 360–363.
17. Periasamy K. Novel antibacterial compounds obtained from some edible mushrooms. *Int J Med Mushrooms* 2005; 7: 443–444.
18. Wang J *et al*. A peptide with HIV-1 reverse transcriptase inhibitory activity from the medicinal mushroom *Russula paludosa*. *Peptides* 2007; 28: 560–565.
19. Blander G *et al*. Superoxide dismutase 1 knock-down induces senescence in human fibroblasts. *J Biol Chem* 2003; 278: 38966–38969.
20. Harman D. Free radical involvement in aging, pathophysiology and therapeutic implications. *Drugs Aging* 1993; 3: 60–80.

21. Liu F *et al*. Free radical scavenging activities of mushroom polysaccharide extracts. *Life Sci* 1997; 60: 763–771.
22. Matsumura Y *et al*. Inhibitory effects of peptide-bound polysaccharide on lipid oxidation in emulsions. *Food Chem* 2003; 83: 107–119.
23. Lyon HO *et al*. Standardized staining methods: feulgen-rossenbeck reaction for desoxyribonucleic acid and periodic acid-Schiff (PAS) procedure. *Biotech Histochem* 2002; 77: 121–125.
24. Dubois M *et al*. Colorimetric method for determination of sugar and related substances. *Anal Chem* 1956; 28: 350–356.
25. Lowry OH *et al*. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; 193: 265–275.
26. Miki M *et al*. Free-radical chain oxidation of rat red blood cells by molecular oxygen and its inhibition by tocopherol. *Arch Biochem Biophys* 1978; 258: 373–380.
27. Sugiyama H *et al*. Purpurogallin as an antioxidant protector of human erythrocytes against lysis by peroxyl radicals. *Life Sci* 1993; 53: 39–43.
28. Liu F, Ng TB. Antioxidative and free radical scavenging activities of selected medical herbs. *Life Sci* 2000; 66: 725–735.
29. Wong JH *et al*. Purification and characterization of a galactose-specific lectin with mitogenic activity from pinto beans. *Biochem Biophys Acta* 2006; 17: 808–813.
30. Collins RA *et al*. A comparison of human immunodeficiency virus type 1 inhibition by partially purified aqueous extracts of Chinese medicinal herbs. *Life Sci* 1997; 60: 345–351.
31. Kim GY *et al*. Purification and characterization of acidic proteoheteroglycan from the fruiting body of *Phellinus linteus* (Berk. & M.A. Curtis) Teng. *Bioresour Technol* 2003; 89: 81–87.
32. Kim YS *et al*. Antiherpetic activities of acidic protein bound polysaccharide isolated from Ganoderma lucidum alone and in combinations with interferons. *J Ethnopharmacol* 2000; 72: 451–458.
33. Oh KW *et al*. Antiherpetic activities of acidic protein bound polysaccharide isolated from *Ganoderma lucidum* alone and in combinations with acyclovir and vidarabine. *J Ethnopharmacol* 2000; 72: 221–227.
34. Slater TF, Cheeseman KH. Free radical mechanisms in relation to tissue injury. *Proc Nutr Soc* 1987; 46: 1–12.
35. Chen Y *et al*. Purification, composition analysis and antioxidant activity of a polysaccharide from the fruiting bodies of *Ganoderma atrum*. *Food Chem* 2008; 107: 231–241.
36. Hirose K *et al*. Induction of gene expression and production of immunomodulating cytokines by PSK in human peripheral blood mononuclear cells. *Lymphokine Res* 1990; 4: 475–483.
37. Liu F *et al*. Induction in the mouse of gene expression of immunomodulating cytokines by mushroom polysaccharide-protein complexes. *Life Sci* 1996; 58: 1795–1803.
38. Richard A *et al*. Polysaccharopeptide from Coriolus versicolor has potential for use against human immunodeficiency virus type 1 infection. *Life Sci* 1997; 60: 383–387.
39. Russell R, Paterson M. Ganoderma A therapeutic fungal biofactory. *Phytochemistry* 2006; 67: 1985–2001.