Glycoconj J (2010) 27:259–265
DOI 10.1007/s10719-009-9274-5

A novel lectin with highly potent antiproliferative and HIV-1 reverse transcriptase inhibitory activities from the edible wild mushroom *Russula delica*

Shuang Zhao · Yongchang Zhao · Shuhong Li · Jingkun Zhao · Guoqing Zhang · Hexiang Wang · Tzi Bun Ng

Received: 11 July 2009 / Revised: 9 December 2009 / Accepted: 9 December 2009 / Published online: 12 January 2010

Abstract A dimeric lectin with a molecular weight of 60 kDa and high hemagglutinating activity was isolated from fresh fruiting bodies of the wild mushroom *Russula delica*. The lectin was composed of two identical subunits, each with a molecular weight of 30 kDa. It was adsorbed on both SP-Sepharose and Q-Sepharose and unadsorbed on DEAE-cellulose. Its hemagglutinating activity was stable up to 70°C, and in HCl and NaOH solutions of concentrations up to 25 and 12.5 mM, respectively. The activity was inhibited by inulin and o-nitrophenyl-β-D-galactopyranoside. Al^{3+}, Fe^{3+} and Zn^{2+} ions, but not by Ca^{2+}, Mg^{2+} and Mn^{2+} ions. Mg^{2+} ions at 10 mM concentration potentiated the hemagglutinating activity of the lectin. *Russula delica* lectin was devoid of mitogenic activity toward mouse splenocytes, but potently inhibited proliferation of HepG2 hepatoma and MCF 7 breast cancer cells, with an IC_{50} value of 0.88 μM and 0.52 μM, respectively. It potently inhibited HIV-1 reverse transcriptase activity with an IC_{50} of 0.26 μM.

Keywords Lectin · Wild mushroom · *Russula delica* · Antiproliferative · Purification

Submitted for publication to Glycoconjugate Journal July 2009.

S. Zhao · J. Zhao · G. Zhang · H. Wang (✉)
State Key Laboratory for Agrobiotechnology and Department of Microbiology, China Agricultural University, Beijing 100094, China
e-mail: hxwang@cau.edu.cn

S. Zhao
Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China

Y. Zhao · S. Li
Institute of Biotechnology and Germplasmic Resource, Yunnan Academy of Agricultural Science, Kunming 650223, China

T. B. Ng (✉)
School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong, China
e-mail: b021770@mailserv.cuhk.edu.hk

Introduction

Lectins are a kind of non-immunogenic proteins or glycoproteins which selectively bind carbohydrates. Many lectins can recognize specific antigens on the surface of cells [1]. They are abundant in animals, plants and microbes including higher fungi and mushrooms [1–3]. Extensive investigations revealed that lectins participate in various biological phenomena such as prevention and/or treatment of cancer, and demonstrate immunomodulatory [4], antiproliferative [5], antitumor/cytotoxic [4, 6], mitogenic [7], and hypotensive [3] activities. Because of these valuable attributes, many investigators pay special attention to lectin research. In recent years, more and more lectins have been isolated from higher mushrooms including *Agaricus bisporus* [5], *Agrocybe cylindracea* [6, 7], *Flammulina velutipes* [8], *Ganoderma lucidum* [9], *Grifola frondosa* [10], *Tricholoma mongolicum* [4], *Volvariella volvacea* [11, 12], and *Schizophyllum commune* [13].

In view of the dearth of information pertaining to proteinaceous components of *Russula delica,* we have devoted our efforts to screen for new lectins in wild mushrooms. We have now isolated a novel lectin from the wild mushroom *Russula delica.* We found that *Russula delica* lectin has potent activities and compared its properties with those of other mushroom lectins.

Materials and methods

Materials

Fresh fruiting bodies of the wild mushroom *Russula delica* were collected in Yunnan province, China. DEAE-cellulose was from Sigma Chemical Company, St. Louis, Missouri, USA. SP-Sepharose and Q-Sepharose were from GE Healthcare, China. The tumor cell lines MCF 7 (breast adenocarcinoma) and HepG2 (hepatoma) were purchased from American Type Culture Collection, USA. All other chemicals used were of analytical grade. HIV-1 reverse transcriptase ELISA kit was from Boehringer-Mannheim (Germany).

Purification of lectin

Fresh *Russula delica* fruiting bodies (600 g) were mashed, homogenized in distilled water, and then left to extract overnight at 4°C. The homogenate was centrifuged at 14,000 g for 25 min. Then, the crude extract was chromatographed on a 5×20 cm column of DEAE-cellulose in 10 mM Tris-HCl buffer (pH 7.2). After removal of the unadsorbed peak D1, adsorbed materials were eluted stepwise with 200 mM NaCl and 1 M NaCl in the same buffer, to yield peaks D2 and D3, respectively. The unadsorbed peak D1, the only peak with hemagglutinating activity, was chromatographed on a 2.5×20 cm column of SP- Sepharose in 10 mM NH_4OAc (AA) buffer (pH 5.2). After elution of the unadsorbed fraction SP1, adsorbed proteins were desorbed using a linear (0–1 M) NaCl gradient. Fraction SP3 with hemagglutinating activity was chromatographed on a 2.5×20 cm column of Q-Sepharose in 10 mM NH_4HCO_3 buffer (pH 9.3). After elution of unadsorbed fraction Q1, adsorbed protein was eluted with a linear 0–1 M NaCl gradient in the same buffer. Hemagglutinating activity was confined to fraction Q3. The active fraction was then dialyzed and applied to an FPLC-Superdex 75 HR10/30 column in 0.2 M NH_4HCO_3 (pH 8.5) using an AKTA Purifier (GE Healthcare). The first peak represented purified lectin.

Molecular weight determination and N-terminal amino acid sequence analysis

The purified lectin was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for molecular weight determination in accordance with the procedure of Laemmli and Favre [14].The N-terminal sequence of *Russula delica* lectin was determined by using a Hewlett-Packard HP G1000A Edman degradation unit and an HP 1000 HPLC System [15].

Assay of hemagglutinating activity

Fifty microliters of a serial twofold dilution of the lectin solution in a microtiter U-plate were mixed with 50 μl of a 2% suspension of rabbit red blood cells in phosphate-buffered saline (pH 7.2) at 20°C.The results were read after about 1 h, when the blank had fully sedimented. The hemagglutination titer, defined as the reciprocal of the highest dilution exhibiting hemagglutination, was reckoned as one hemagglutination unit. Specific activity is the number of hemagglutination units/mg protein [15].

Effects of various carbohydrates on hemagglutination activity

Serial twofold dilutions of sugar samples were prepared in phosphate buffered saline (PBS). The different dilutions of a sugar were mixed with an equal volume (25 μl) of a solution of the lectin with 8 hemagglutination units. The mixture was allowed to stand for 30 min at room temperature, and then mixed with 50 μl of a 2% rabbit erythrocyte suspension. The minimum concentration of the sugar in the final reaction mixture, which completely inhibited 8 hemagglutination units of the lectin preparation, was calculated [16].

Effects of temperature on hemagglutinating activity

Aliquots of the lectin solution were kept separately at different temperatures (10°C to 100°C, at 10°C intervals) for 30 min. Then the hemagglutination activity was determined [16].

Effects of HCl and NaOH solutions on hemagglutinating activity

Aliquots of the lectin solution were preincubated separately in 0.0125 M, 0.025 M, 0.05 M, 0.1 M, 0.2 M HCl or NaOH solutions. Two hours later, an equal volume of NaOH or HCl solution was added to achieve neutralization. The hemagglutinating activity was determined. The control group was treated with phosphate buffer saline [16].

Effects of cations on hemagglutinating activity

The lectin sample was demetalized by dialysis exhaustively against 10 mM EDTA, and the hemagglutination activity was then determined. The treated lectin was incubated with one of the cations (Table 4) for 2 h, and then the hemagglutination activity was examined [15].

Assay of antiproliferative activity on tumor cell lines

The antiproliferative activity of the purified lectin was determined as follows. MCF-7 (breast adenocarcinoma) and HepG2 (hepatoma) cells were cultured in RPMI medium supplemented with 10% (v/v) fetal bovine serum (FBS), 100 mg/l streptomycin, and 100 IU/ml penicillin at 37°C in a humidified atmosphere of 5% (v/v) CO_2. Cells were subsequently seeded into 96-well plates at a density of 2×10^3 cells/well, and incubated for 24 h. Different concentrations of the lectin in 100 μl complete RPMI medium were then added to the wells and incubated for 72 h. After that, [3-[4, 5-dimethythiazol-2-yl]-2,5-diphenyltetrazolium bromide] (MTT) quantification assay was carried out to measure viability of the cells. Briefly, 20 μl of a 5 mg/ml MTT solution in phosphate buffered saline was spiked into each well and the plates were incubated for 4 h. The plates were then centrifuged at 2,500 rpm for 5 min. The supernatant was carefully removed, and 150 μl of dimethyl sulfoxide was added in each well to dissolve the MTT-formazan formed at the bottom of the wells. After 10 min, absorbance at 590 nm was measured with a microplate reader. As a control, PBS instead of lectin was added into the wells [17].

Assay of mitogenic activity of lectin toward mouse splenocytes

The assay was performed as described by Wang *et al*. [7]. Splenocytes were isolated from BALB/c mice. The cell suspension was diluted with RPMI medium containing 10% fetal bovine serum and then seeded (2×10^6cells/0.2 ml/well) in 96-well microplates. The lectin was then added at various concentrations. Cells cultured in the absence of lectin served as control. The cells were incubated at 37°C in a humidified atmosphere of 5% carbon dioxide for 24 h. They were found to be viable after 24 h. During the last 6 h, the cells in one well were pulsed with 0.5 μCi [^{3}H-methyl] thymidine (specific activity 5 μCi/mmol, GE Healthcare) in 10 μl and then harvested onto a glass fiber filter using a cell harvester. The radioactivity was determined using a Beckman scintillation counter. The proliferative (mitogenic) response was expressed as mean counts per min (cpm).

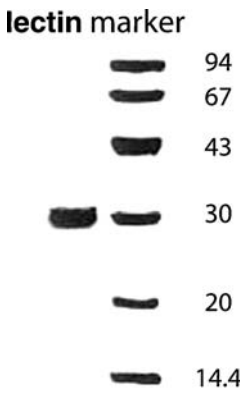

Fig. 1 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *Left lane*: *Russula delica* lectin. *Right lane*: molecular mass standards, from *top downward*: phosphorylase b (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa),carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20 kDa), and α-lactalbumin, (14.4 kDa)

Table 1 Yields and hemagglutinating activities of various chromatographic fractions (from 600 g fresh *Russula delica* fruiting bodies)

Chromatographic fraction	Yield (mg)	Specific hemagglutinating activity (Titer/mg)	Purification fold
Extract	1,975.4	218	1.0
D1	**473.5**	**658**	**3.0**
D2	440.1	120	0.6
D3	509.7	<40	–
SP1	40.2	<40	–
SP2	93.7	<40	–
SP3	**80.0**	**2,820**	**12.9**
SP4	120.5	170	0.8
Q1	14.2	<40	–
Q2	20.5	<40	–
Q3	**22.1**	**7,160**	**32.8**
SU1	**6.3**	**17,180**	**78.8**
SU2	2.9	220	1.0
SU3	5.8	<40	–

The lectin-enriched fractions are shown in bold

Assay for HIV reverse transcriptase (HIV RT) inhibitory activity

The assay for HIV RT inhibitory activity was performed, as described by Han *et al*. [13], using a non-radioactive enzyme-linked immunosorbent assay (ELISA) kit from Boehringer-Mannheim (Germany). The assay was carried out as stated in the protocol that came with the kit, except that each well contained 2 ng of recombinant HIV-1 reverse transcriptase in a total reaction volume of 60 μl. It made use of the ability of reverse transcriptase to synthesize DNA, starting from the template/primer hybrid poly(A)-oligo(dT)15. In this assay, nucleotides labeled with digoxigenin and biotin in an optimized proportion were incorporated into the DNA, which was freshly synthesized by the reverse transcriptase (RT). The detection and quantification of synthesized DNA as a parameter for RT activity follows a sandwich ELISA protocol. The surface of the microtiter plate modules, which had been coated with streptavidin, allowed the binding of biotin-labeled DNA. An antibody, which had been conjugated to

Table 2 Comparison of N-terminal sequences of lectins from *Russula delica*, *Armillaria luteo-virens* and *Pleurotus cornucopiae*

Russula delica lectin	GLK · LAKQFA · L
Armillaria luteo-virens lectin	G · · · · ·AKQTKWIV
Pleurotus cornucopiae lectin	· MKTLAW · FVPL

_: Identical corresponding amino acids are underlined

·: Space created to maximize sequence similarity

peroxidase, to digoxigenin (anti-DIG-POD) was then used to bind to the digoxigenin-labeled DNA. This was followed by the addition of peroxidase substrate. The peroxidase enzyme, conjugated to the antibody, then catalysed cleavage of the substrate to produce a coloured product. The absorbance was then measured at 405 nm with a microtiter (ELISA) reader and then correlated to the level of RT activity. A fixed amount (4–6 ng) of recombinant HIV-1 reverse transcriptase was used. The activity of inhibition exhibited by the lectin was determined as compared to a control without lectin.

Results

Purification of *Russula delica* lectin

Hemagglutinating activity was detected only in the unadsorbed fraction D1 from the DEAE- cellulose column, Fraction SP3 eluted with a linear 0–1 M NaCl from the SP-Sepharose column contained hemagglutinating activity. Fraction Q3 eluted with a linear 0–1 M NaCl gradient from Q-Sepharose was the only fraction with hemagglutinating activity. Q3 was resolved on Superdex 75 into three peaks: SU1, SU2 and SU3. The first peak SU1, but not the second and third peaks, showed hemagglutinating activity. The molecular weight of SU1, as judged from its elution volume from the Superdex 75 gel filtration column, was 60 kDa. SU1 appeared as a single 30-kDa band in SDS-polyacrylamide gel electrophoresis, indicating that it was a dimer, and that the MW of the subunits was 30 kDa (Fig. 1). The yields and specific hemagglutinating activities of the various chromatographic fractions are given in Table 1. The N-terminal sequence of the lectin was GLKLAKQFAL. It demonstrated some similarity to lectins from *Armillaria luteo-virens* and *Pleurotus cornucopiae* (Table 2).

Characterization of *Russula delica* lectin

Russula delica lectin was stable from 10°C to 70°C. Its hemagglutination activity declined to 50% of the initial activity at 80°C, to 12.5% of the initial activity at 90°C, and to negligible levels at 100°C (Table 3). The lectin was stable in 0.025 M HCl and 0.0125 M NaOH, while it was completely destroyed in 0.1 M HCl and NaOH. In 0.05 M HCl and NaOH, the activity declined to 25% of the initial activity (Table 4). The hemagglutination activity was not affected by treatment with $Ca^{2+}$and Mn^{2+} ions. However, the activity could be increased by adding 10 mM Mg^{2+} and decreased by adding Al^{3+}, Fe^{3+} and Zn^{2+} ions (Table 5). Inulin and o-nitrophenyl-β-D-galactopyranoside inhibited the activity of *Russula delica* lectin, with the minimum inhibitory concentration of 50 mM and 25 mM, respectively (Table 6).

Proliferation of HepG2 and MCF-7 cancer cells was effectively inhibited by the lectin. When the lectin concentration was 2 μM, the inhibition ratio achieved was 71.1% and 90.4%, respectively. The lectin inhibited HepG2 and MCF-e7 cells with an IC_{50} value of 0.88 μM and 0.52 μM, respectively (Fig. 2). At 0.08 μM, 0.4 μM and 2 μM, the lectin inhibited HIV-1 reverse transcriptase by 23.8%, 60.5% and 94.2%, respectively. The IC_{50} was estimated to be 0.26 μM. The lectin lacked mitogenic activity toward mouse splenocytes (Fig. 3).

Discussion

Russula delica lectin is a homodimeric lectin with a molecular mass of 60 kDa. The N-terminal sequence of this lectin, GLKLAKQFAL, has not been reported for other

Table 3 Effect of temperature on hemagglutinating activity of *Russula delica* lectin

Initial hemagglutinating activity was 16 hemagglutinating units

Temperature (°C)	10	20	30	40	50	60	70	80	90	100
Hemagglutinating activity (U)	16	16	16	16	16	16	16	8	2	0

Table 4 Effect of NaOH and HCl solution on hemagglutinating activity of *Russula delica* lectin

Initial hemagglutinating activity was 16 hemagglutinating units

HCl (M)	0.0125	0.025	0.05	0.1	0.2
Hemagglutinating activity (U)	16	16	4	0	0
NaOH (M)	0.0125	0.025	0.05	0.1	0.2
Hemagglutinating activity (U)	16	8	4	0	0

Table 5 Effects of cations on hemagglutinating activity of *Russula delica* lectin

Cation	Concentration(mM)			
	10	5	2.5	1.25
Mg^{2+}	32	16	16	16
Mn^{2+}	16	16	16	16
Ca^{2+}	16	16	16	16
Zn^{2+}	0	0	8	8
Al^{3+}	0	0	8	8
Fe^{3+}	0	0	0	8

Initial hemagglutinating activity was 16 hemagglutinating units

lectins. It was stable at the moderately high temperature of 70°C and it could recognize the structures of both o-nitrophenyl-β-D-galacto-pyranoside and inulin. The lectin purified from *Russula delica* is dimeric, like lectins from mushrooms including *Schizophyllum commune* [13], *Armillaria luteo-virens* [18], *Agrocybe cylindracea* [7], *Coprinus cinereus* [19] and *Oudemansiella platyphylla* [20]. However, it is unlike monomeric *Flammulina velutipes* lectin [8] and tetrameric *Hericium erinaceum* lectin [21]. The isolated lectin is similar in N-terminal sequence to two other Agaricales lectins, *Armillaria luteo-virens* lectin and *Pleurotus cornucopiae* lectin. The protocol used was similar to the isolation procedures previously employed for other mushroom lectins [7, 12, 13, 16, 18], It comprised anionic and cationic exchange chromatography and gel filtration. The lectin was unadsorbed on DEAE-cellulose, but adsorbed on SP-Sepharose and Q-Sepharose (Table 7).

The antiproliferative activity of *Russula delica* lectin is in agreement with previous reports on lectins from the mushrooms *Agaricus bisporus* [3, 5], *Tricholoma mongolicum* [4], *Schizophyllum commune* [13] and *Pleurotus ostreatus* [16]. The lectin exerts a potent antiproliferative effect on HepG2 and MCF7 cells, with an IC_{50} value of 0.88 μM and 0.52 μM, respectively. This activity is extremely potent, with an IC_{50} at nanomolar concentrations. Compared with many other lectins, *Russula delica* lectin has a significant inhibitory activity toward HIV-1 reverse transcriptase, with an IC_{50} value of 0.26 μM. It is possible that the mechanism of inhibition is analogous to the protein-protein interaction involved in the inhibition of HIV-1reverse transcriptase by the homologous protease [22]. The potency of inhibition (<1 μM) is high compared with proteinaceous and non proteinaceous anti-HIV natural products. It remains to be seen whether *Russula delica* lectin can be applied in the areas of cancer therapy, AIDS and immunoinsufficiency. *Russula delica* lectin is devoid of mitogenic activity toward mouse splenocytes, in contrast to lectins such as those from *Agrocybe cylindracea* [7] and *Tricholuma mongolicum* [4, 17].

Russula delica lectin is unstable at temperatures above 70°C, and also at concentrations of HCl above 25 mM and concentrations of NaOH above 12.5 mM. It has relatively high thermostability since other mushroom lectins [23, 24], except a few like *Ganoderma capense* lectin [25], cease to be stable at a lower temperature. The hemagglutinating activity of *Russula delica* lectin is attenuated in the presence of cations including Al^{3+}, Fe^{3+} and Zn^{2+} ions and carbohydrates of inulin and o-nitrophenyl-β-D-galactopyranoside. The attenuation of the hemagglutinating activity of the lectin by Al^{3+} and Zn^{2+} ions is in contrast to the potentiation of the hemagglutinating activity of *Xerocomus spadiceus* lectin by the same ions [15]. The

Table 6 Effect of various carbohydrates on hemagglutination activity of *Russula delica* lectin

Sugar (mM)	0.78	1.56	3.12	6.25	12.5	25	50	100	200
Inositol	+	+	+	+	+	+	+	+	+
o-Nitrophenyl-β-D-galactopyranoside	+	+	+	+	+	–	–	–	–
L(−)Sorbose	+	+	+	+	+	+	+	+	+
Raffinose	+	+	+	+	+	+	+	+	+
L(+)-Rhamnose	+	+	+	+	+	+	+	+	+
D(−)-Fructose	+	+	+	+	+	+	+	+	+
D(−)-Mannose	+	+	+	+	+	+	+	+	+
Cellobiose	+	+	+	+	+	+	+	+	+
L(+)-Arabinose	+	+	+	+	+	+	+	+	+
D(+)-Xylose	+	+	+	+	+	+	+	+	+
D-Melibiose	+	+	+	+	+	+	+	+	+
α-Lactose	+	+	+	+	+	+	+	+	+
Inulin	+	+	+	+	+	+	–	–	–
Maltose	+	+	+	+	+	+	+	+	+
D(+)-Galactose	+	+	+	+	+	+	+	+	+
D-Glucose	+	+	+	+	+	+	+	+	+

Initial hemagglutinating activity was 8 hemagglutinating units, +, hemagglutination, −, no hemagglutination

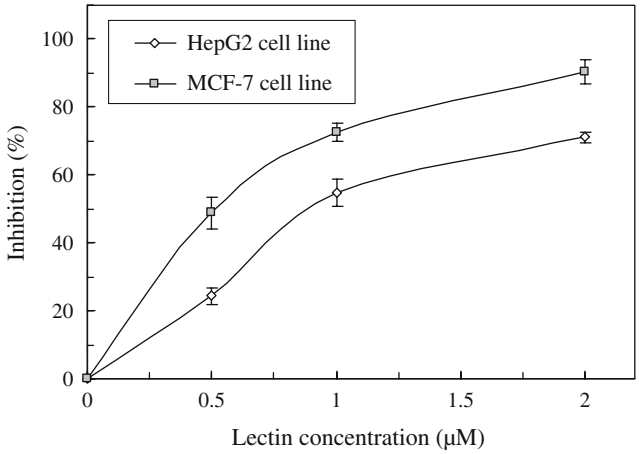

Fig. 2 Antiproliferative activities of *Russula delica* lectin towards HepG2 and MCF7 cells. Results represented means ± SD(n=3). IC_{50} value, the concentration of lectin that results in an inhibition ratio of 50%, is 0.88 μM for HepG2 cells and 0.52 μM for MCF7 cells

potentiation of its hemagglutinating activity by Mg^{2+} ions is reminiscent of a similar observation on Con A. However, the activity of Con A is also enhanced by Ca^{2+} and Mn^{2+} ions, whereas that of *Russula delica* lectin remains unaffected. Mushroom lectins can be divided into different groups, with each group having different carbohydrate-binding specificities. For instance, one group can be inhibited by glycoproteins and simple sugars, one inhibited by glycoproteins but not by simple sugars, and another one not inhibited by glycoproteins or simple sugars. Among the various carbohydrates tested, inulin and o-nitrophenyl-β-D-galactopyranoside were the only sugars capable of inhibiting the hemagglutinating activity of *Russula delica* lectin, and other sugars tested had no inhibitory effects on the hemagglutinating activity of the lectin. This indicated that the structure of *Russula delica* lectin is specific for the

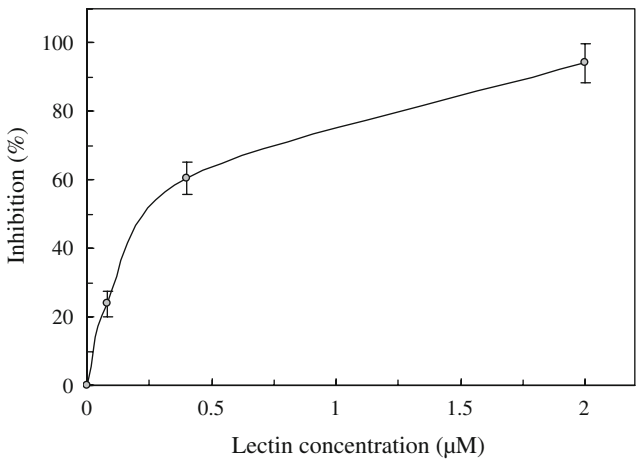

Fig. 3 The inhibitory activity of *Russula delica* lectin towards HIV-1 reverse transcriptase. Results represented means ± SD(n=3). IC_{50} value, the concentration of lectin that results in an inhibition ratio of 50%, is 0.26 μM

Table 7 Comparison of characteristics of lectins from *Russula delica* and other fungi

	Russula delica	*Cordyceps militaris* [27]	*Pholiota adiposa* [28]	*Inocybe umbrinella* [29]	*Boletus edulis*[30]	*Pleurotus citrinopileatus*[31]	*Armillaria luteo-virens* [18]
N-terminal sequence	GLKLAKQFAL	NSTDISLNHG	DILMGTYGML	DGVLATNAVA	TYGIALRV	QYSQMAQVME	GVVFFAAFKQTKWIV
Molecular mass (kD)	60	30	32	17	32.6	32.4	29.4
Carbohydrate specificity	Inulin, o-nitrophenyl-β-D-galactopyranoside	–	Inulin	Raffinose, D-melibiose, α-lactose, D-galactose	D(+)-melibiose D-xylose	Maltose, o-nitrophenyl-beta-D-galactopyranoside, O/P-nitrophenyl-beta-D-glucuronide, Insulin	Inulin
Thermal stability(°C)	70	60	50	60	60	60	70
Chromatographic behavior on							
(i) DEAE-ion exchanger	Unadsorbed	–	Adsorbed	Adsorbed	Unadsorbed	Unadsorbed	Unadsorbed
(ii) Q- ion exchanger	Adsorbed	Adsorbed	–	–	–	Adsorbed	Adsorbed
(iii) Cation exchanger	Adsorbed	–	Adsorbed	Adsorbed	Adsorbed	Adsorbed	Adsorbed
(iv) Affi-gel	–	Unadsorbed	–	–	Unadsorbed	–	–

– means not determined or not attempted

[18], [27–31]: Reference number

structures of inulin and o-nitrophenyl-β-D-galactopyranoside. Thus, immobilized *Russula delica* lectin can be used as an affinity chromatography media for interactions with molecules containing these sugars. In comparison, the carbohydrate specificity of *Russula delica* lectin is similar to that of *Pleurotus citrinopileatus,* but different from the previously reported carbohydrate specificities of *Cordyceps militaris, Inocybe umbrinella* and *Boletus edulis* which were [27, 29, 30] (Table 7).

There are only four publications concerning *Russula delica* in the literature. Literature on proteins from the mushroom *R. delica* is completely lacking. The present report is an addition to the meager literature. In summary, *Russula delica* lectin has its unique features which provide possibilities for application in antitumor therapy. Future research work may include drug delivery investigation like antitumor drug modification [26].

Acknowledgments This work was financially supported by National Grants of China (nyhyzx07-008 and 2006BAD07A01).

References

1. Guillot, J., Konska, G.: Lectins in higher fungi. Biochem. Syst. Ecol. **25**, 203–230 (1997)
2. Ng, T.B.: Peptides and proteins from fungi. Peptides **25**, 1055–1073 (2004)
3. Wang, H.X., Ng, T.B., Ooi, V.E.C.: Lectins from mushrooms. Mycol. Res. **102**, 897–906 (1998)
4. Wang, H.X., Liu, W.K., Ng, T.B.: The immunomodulatory and antitumor activities of lectins from mushroom *Tricholoma mongolicum*. Immunopharmacology **31**, 205–211 (1996)
5. Yu, L.G., Fernig, D.J., Smith, J.A., Milton, J.D., Rhodes, J.M.: Reversible inhibition of proliferation of epithelial cell lines by *Agaricus bisporus* (edible mushroom) lectin. Cancer Res. **53**, 4627–4632 (1993)
6. Yagi, F., Miyamoto, M., Abe, T., Minami, T., Tadera, K., Goldstein, I.J.: Purification and carbohydrate-binding specificity of *Agrocybe cylindracea* lectin. Glycoconj. J. **14**, 281–288 (1997)
7. Wang, H.X., Ng, T.B., Liu, Q.H.: Isolation of a new heterodimeric lectin with mitogenic activity from fruiting bodies of the mushroom *Agrocybe cylindracea*. Life Sci. **70**, 877–886 (2002)
8. Ko, J.L., Hsu, C.I., Lin, R.H., Kao, C.L., Lin, J.Y.: A new fungal immunomodulatory protein, FIP-fve isolated from the edible mushroom. Flammulina velutipes and its complete amino acid sequence. Eur. J. Biochem. **228**, 244–249 (1995)
9. Kawagishi, H., Mitsunaga, S.I., Yamawaki, M., Ido, M., Shimada, A., Kinoshita, T., Murata, T., Usui, T., Kimura, A., Chiba, S.: A lectin from mycelia of the fungus *Ganoderma lucidum*. Phytochemistry **44**, 7–10 (1997)
10. Kawagishi, H., Nomura, A., Mizuno, T., Kimura, A., Chiba, S.: Isolation and characterization of a lectin from *Grifola frondosa* fruiting bodies. Biochim. Biophys. Acta **1034**, 247–252 (1990)
11. Lin, J.Y., Chou, T.B.: Isolation and characterization of a lectin from edible mushroom Volvariella volvacea. J. Biochem. **96**, 35–40 (1984)
12. She, Q.B., Ng, T.B., Liu, W.K.: A novel lectin with potent immunomodulatory activity isolated from both fruiting bodies and cultured mycelia of the edible mushroom *Volvariella volvacea*. Biochem. Biophys. Res. Commun. **247**, 106–111 (1998)
13. Han, C.H., Liu, Q.H., Ng, T.B., Wang, H.X.: A novel homodimeric lactose-binding lectin from the edible split gill medicinal mushroom *Schizophyllum commune*. Biochem. Biophys. Res. Commun. **336**, 252–257 (2005)
14. Laemmli, U.K., Favre, M.: Maturation of the head of bacteriophage T4. J. Mol. Biol. **80**, 575–599 (1973)
15. Liu, Q.H., Wang, H.X., Ng, T.B.: Isolation and characterization of a novel lectin from the wild mushroom *Xerocomus spadiceus*. Peptides **25**, 7–10 (2004)
16. Wang, H.X., Gao, J., Ng, T.B.: A new lectin with highly potent antihepatoma and antisarcoma activities from the oyster mushroom *Pleurotus ostreatus*. Biochem. Biophys. Res. Commun. **275**, 810–816 (2000)
17. Wang, H.X., Ng, T.B., Liu, W.K., Ooi, V.E.C., Chang, S.T.: Isolation and characterization of two distinct lectins with antiproliferative activity from the cultured mycelium of the edible mushroom *Tricholoma mongolicum*. Int. J. Pept. Protein Res. **46**, 508–513 (1995)
18. Feng, K., Liu, Q.H., Ng, T.B., Liu, H.Z., Li, J.Q., Chen, G., Sheng, H.Y., Xie, Z.L., Wang, H.X.: Isolation and characterization of a novel lectin from the mushroom *Armillaria luteo-virens*. Biochem. Biophys. Res. Commun. **345**, 1573–1578 (2006)
19. Cooper, D.N., Boulianne, R.P., Charlton, S., Fanell, E.M., Sucher, A., Lum, B.C.: Fungal galectins, sequence and specificity of two isolectins from *Coprinus cinereus*. J. Biol. Chem. **272**, 1514–1521 (1997)
20. Matsumoto, H., Natsume, A., Ueda, H., Saitoh, T., Ogawa, H.: Screening of a unique lectin from 16 cultivable mushrooms with hydrid glycoprotein and neoproteoglycan probes and purification of a novel N-acetylglucosamine-specific lectin from *Oudemansiella platyphylla* fruiting body. Biochim. Biophys. Acta **1526**, 37–43 (2001)
21. Kawagishi, H., Mori, H., Uno, A., Kimura, A., Chiba, S.: A sialic acid binding lectin from the mushroom *Hericium erinaceum*. FEBS Lett. **340**, 56–58 (1994)
22. Bottcher, M.: Grosse, F.: HIV-1 protease inhibits its homologous reverse transcriptase by protein-protein interaction. Nucleic Acids Res. **25**, 1709–1714 (1997)
23. Jung, E.C., Kim, K.D., Kim, H.H.: A mushroom lectin from ascomycete *Cordyceps militaris*. Biochim. Biophys. Acta **1770**, 833–838 (2007)
24. Liu, Q.H., Wang, H.X., Ng, T.B.: First report of a xylose-specific lectin with potent hemagglutinating, antiproliferative and antimitogenic activities from a wild ascomycete mushroom. Biochim. Biophys. Acta **1760**, 1914–1919 (2006)
25. Patrick, H.K., Ng, T.B.: A mushroom (*Ganoderma capense*) lectin with spectacular thermostability, potent mitogenic activity on splenocytes, and antiproliferative activity toward tumor cells. Biochem. Biophys. Res. Commun. **314**, 988–993 (2004)
26. Dong, Y.C., Feng, S.S.: Poly(d,l-lactide-*co*-glycolide) (PLGA) nanoparticles prepared by high pressure homogenization for paclitaxel chemotherapy. Int. J Pharm **342**, 208–214 (2007)
27. Wong, J.H., Wang, H.X., Ng, T.B.: A haemagglutinin from the medicinal fungus *Cordyceps militaris*. Biosci. Rep. **29**, 321–327 (2009)
28. Zhang, G.Q., Sun, J., Wang, H.X., Ng, T.B.: A novel lectin with antiproliferative activity from the medicinal mushroom *Pholiota adiposa*. Acta Biochim. Pol. **56**, 415–421 (2009)
29. Zhao, J.K., Wang, H.X.: Ng, T.B.:Purification and characterization of a novel lectin from the toxic wild mushroom Inocybe umbrinella. Toxicon. **53**, 360–366 (2009)
30. Zheng, S.Y., Li, C.X., Ng, T.B., Wang, H.X.: A lectin with mitogenic activity from the edible wild mushroom *Boletus edulis*. Process Biochem. **42**, 1620–1624 (2007)
31. Li, Y.R., Liu, Q.H., Wang, H.X., Ng, T.B.: A novel lectin with potent antitumor, mitogenic and HIV-1 reverse transcriptase inhibitory activities from the edible mushroom *Pleurotus citrinopileatus*. Biochim. Biophys. Acta **1780**, 51–57 (2008)