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A novel lectin with potent antitumor, mitogenic and HIV-1 reverse transcriptase inhibitory activities from the edible mushroom *Pleurotus citrinopileatus*

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Abstract

The objective of the present study was to isolate a lectin from fresh fruiting bodies of the mushroom *Pleurotus citrinopileatus* and examine it for various biological activities. The isolation procedure comprised ion exchange chromatography on DEAE-cellulose, CM-celluloses, and Q-Sepharose, and gel filtration on Superdex 75. A homodimeric 32.4 kDa lectin displaying high hemagglutinating activity was isolated with over 110 fold of purification. Its N-terminal amino acid sequence, QYSQMAQVME, has not been reported for other lectins. The lectin was unadsorbed on DEAE-cellulose in 0.001 M NH_4HCO_3 buffer (pH 9.4), but adsorbed on CM-cellulose in 0.001 M NH_4OAc buffer (pH 4.8) and eluted by approximately 0.05 M NaCl in the same buffer. The lectin was subsequently adsorbed on Q-Sepharose and eluted by a linear gradient of 0–0.2 M NaCl in 10 mM NH_4HCO_3 buffer (pH 8.5). The lectin was obtained in a purified form after gel filtration by fast protein liquid chromatography on Superdex 75. The hemagglutinating activity of the lectin was inhibited by maltose, *O*-nitrophenyl-β-D-galactopyranoside, O/P-nitrophenyl-β-D-glucuronide and insulin. It was stable at temperatures up to 60 °C, and in NaOH and HCl solutions up to 0.1 M and 0.006 M concentration, respectively. It was sensitive to inhibition by $HgCl_2$ and potentiation by $AlCl_3$. The lectin exerted potent antitumor activity in mice bearing sarcoma 180, and caused approximately 80% inhibition of tumor growth when administered intraperitonealy at 5 mg/kg daily for 20 days. It elicited a mitogenic response from murine splenocytes *in vitro* with the maximal response at a lectin concentration of 2 μM. The lectin inhibited HIV-1 reverse transcriptase with an IC_{50} of 0.93 μM. It was devoid of antifungal activity.

Keywords: Lectin; *Pleurotus citrinopileatus*; Hemagglutinating activity; Antitumor activity; Mitogenic activity; HIV-1 reverse transcriptase inhibitory activity

1. Introduction

Lectins are carbohydrate-binding proteins found in a variety of organisms, including animals, plants, fungi, bacteria and viruses [1,2]. These proteins display a diversity of important biological activities including antimicrobial, anticancer, immunomodulatory and anti-insect activities.

Mushrooms have captured the attention of some investigators on account of the exploitable bioactive constituents that they contain. Mushroom polysaccharides may exhibit hypolipidemic, hypoglycemic, immunostimulant and antitumor activities [3,4]. Mushroom polysaccharide-peptides and polysaccharide–protein complexes may exert immunomodulatory and antitumor actions [5–7]. Some mushroom lectins are endowed with antiproliferative, antitumor, mitogenic, hypotensive, vasorelaxing, hemolytic, anti-HIV-1 reverse transcriptase and immunoenhancing activities [8–23]. Ribosome inactivating proteins, antifungal proteins [24,25], enzymes [26–30], and ubiquitin-like proteins [31] have also been isolated.

Different amino acid sequences have been reported for different mushroom lectins [8–11,14,32,33]. In view of the importance of lectins, the isolation of a new mushroom lectin is a worthwhile undertaking. *Pleurotus citrinopileatus* is a cultivated mushroom. Few studies, however, have been conducted on this species. An alkaline protease [34], and polysaccharides with antihyperglycemic [35], and antitumor [36,37] activities have

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been reported from this mushroom. The mushroom can also decolorize and degrade the commercial reactive dye blue-BF-R [38] and is potentially useful for treatment of effluents containing this dye. Both methanolic and ethyl acetate extracts of this mushroom exhibited antihyperlipidemic and antioxidant activities [39]. The purpose of the present study was to isolate a lectin from this edible mushroom, and to compare its physicochemical and biological characteristics with other mushroom lectins to see if there are any outstanding features and potentially exploitable properties. It was revealed that *P. citrinopileatus* lectin exhibited some distinctive characteristics and potentially exploitable activities.

2. Materials and methods

2.1. Purification scheme

Fresh fruiting bodies (150 g) of the mushroom *P. citrinopileatus* collected from Yunnan Province in China were homogenized in 0.15 M NaCl (5 ml/g) using a Waring blender, and then soaked in 0.5 L of 0.15 M NaCl for 12 h. The slurry was centrifuged at 8000×*g* for 25 min. $(NH_4)_2SO_4$ was added to the supernatant to attain 30%–80% saturation. The precipitate was collected by centrifugation (8000×*g*, 4 °C, 25 min), dissolved in a small amount of distilled water, and dialyzed extensively against 0.01 M NH_4HCO_3 buffer (pH 9.4). The crude extract was then applied to a column of DEAE-cellulose column (Sigma) (2.5×0 cm) which had previously been equilibrated with and was then eluted with 0.01 M NH_4HCO_3 buffer (pH 9.4). Following elution of the unadsorbed fraction D1 with 6 bed volumes of buffer, fractions D2 and D3 were obtained by eluting the column with 5 bed volumes of 0.05 M NaCl and 0.15 M NaCl in the NH_4HCO_3 buffer, respectively. The unadsorbed fraction (D1) was subjected to cation exchange chromatography on a CM-cellulose(Sigma)column (2.5×20 cm) in 0.01 M NH_4OAc buffer (pH 4.8). After removal of the unadsorbed fraction C1 with 3 bed volumes of 0.01 M NH_4OAc buffer (pH 4.8), the column was eluted with 3 bed volumes of 0.05 M NaCl and 0.15 M NaCl in the NH_4OAc buffer to yield fractions C2 and C3, respectively. Fraction C2 was further fractionated by anion exchange chromatography on a Q-Sepharose (Amersham Biosciences) column (1.0×10 cm). After the unadsorded fraction Q1 had been eluted with 0.01 M NH_4HCO_3 buffer (pH 8.6), the adsorbed fractions were eluted with a linear gradient of 0–0.6 M NaCl in the same buffer. Fraction Q3 was subjected to gel filtration on a Superdex 75 HR 10/30 column by fast protein liquid chromatography using an AKTA Purifier (Amersham Biosciences). The major peak obtained constituted purified lectin.

2.2. Determination of molecular mass and N-terminal sequence

The purified lectin was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for molecular mass determination in accordance with the procedure of Laemmli and Favre [40]. Gel filtration on an FPLC-Superdex 75 column, which had been calibrated with molecular mass markers (Amersham Biosciences), was conducted to determine the molecular mass of the lectin. The N-terminal sequence of the lectin was determined by using a Hewlett-Packard HP G1000A Edman degradation unit and an HP 1000 HPLC System [24].

2.3. Assay of hemagglutinating activity

In the assay for lectin (hemagglutinating) activity, a serial two-fold dilution of the lectin solution in microtiter U-plates (50 μl) was mixed with 50 μl of a 2% suspension of rabbit red cells in phosphate-buffered saline (pH 7.2) at 20 °C. The results were recorded after about 1 h, when erythrocytes in the blank had fully sedimented. The hemagglutination titer, defined as the reciprocal of the highest dilution of the lectin solution exhibiting hemagglutination, was reckoned as one hemagglutination unit. Specific activity is the number of hemagglutination units per mg protein [8].

The hemagglutination inhibition tests to investigate inhibition of lectin-induced hemagglutination by various carbohydrates were performed in a manner analogous to the hemagglutination test. The carbohydrates tested included insulin, D(+)-melibiose, D-fructose, L-arabinose, D-arabinose, L-rhamnose, D-xylose, sorbose, inositol, lactose, D-galactose, sorbose, adonitol, cellobiose, glucose, D-mannose, raffinose, turanose, sucrose, maltose, and dulcitol [8].

Serial twofold dilutions of sugar samples were prepared in phosphate buffered saline. All of the dilutions were mixed with an equal volume (25 μl) of a solution of the lectin with 32 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 32 hemagglutination units of the lectin preparation, was calculated [8].

The effects of NaOH,HCl, metal chlorides (including those of heavy metals in order to examine a possible inactivation by heavy metals due to their effect on the SH groups), and temperature on hemagglutinating activity of the lectin were carried out as previously described in [8].

2.4. Assay of antifungal activity

The assay for antifungal activity toward various fungal species including *Botrytis cinerea*, *Fusarium oxysporum*, *Mycosphaerella arachidicola* and *Physalospora piricola* was carried out in petri plates (100×15 mm) containing 10 ml of potato dextrose agar. After the mycelial colony had developed, sterile blank paper disks (0.625 cm in diameter) were placed at a distance of 0.5 cm away from the rim of the mycelial colony. An aliquot (15 μl) of the lectin containing 30 μg, 60 μg or 120 μg was added to a disk. The plates were incubated at 23 °C (temperature optimal for the growth of the fungi) for 72 h until mycelial growth had enveloped disks containing the control and had formed crescents of inhibition around disks containing samples with antifungal activity [24,41]. The mushroom antifungal protein lyophyllin with activity against the aforementioned fungi [24] was used as a positive control.

Animal experiments described below were approved by Animal Research Ethics Committee, The Chinese University of Hong Kong and carried out in accordance with their guidelines.

2.5. Assay for antitumor activity

2.5.1. Inhibition of tumor growth

An ICR mouse was sacrificed on the seventh day after inoculation of sarcoma 180 tumor cells, and the ascitic fluid was collected. After the cells had been washed three times with phosphate-buffered saline, the cell suspension was diluted to 5×10^6 cells/ml, and 0.1 ml of the suspension was subcutaneously inoculated into the inguinal area of the ICR mouse. After inoculation, the mice received daily intraperitoneal injections of the lectin at the dose of 5 mg/kg body weight/day for 20 days. The control group was treated with phosphate-buffered saline (pH 7.2), daily for 20 days. On the twenty-first day after inoculation, the tumor was excised and weighed. The antitumor activity was calculated as

$$\%\ \text{Tumor inhibition} = (C - T)/C \times 100\%$$

where C is the average tumor weight of control group and T is the average tumor weight of lectin-treated group [7,12].

2.6. Assay for mitogenic activity toward mouse splenocytes

The assay was conducted as described in [6,7,10,12].

The assay was approved by the Animal Research Ethics Committee, The Chinese University of Hong Kong and performed in compliance with its regulations. Four C57BL/6 mice (20–25 g) were sacrificed by cervical dislocation and the spleens were aseptically removed. Splenocytes were isolated by pressing the tissue through a sterilized 100-mesh stainless steel sieve and resuspended to 5×10^6 cells/ml in RPMI 1640 culture medium supplemented with 10% fetal bovine serum, 100 units penicillin/ml, and 100 μg streptomycin/ml. The splenocytes (7×10^5 cells/100 μl/well) were seeded into a 96-well culture plate and serial concentrations of the lectin in 100 μl medium were added. Following incubation of the splenocytes at 37 °C in a humidified atmosphere of 5% CO_2 in the presence or absence of the lectin for 24 h, 10 μl [methyl ^{3}H]-

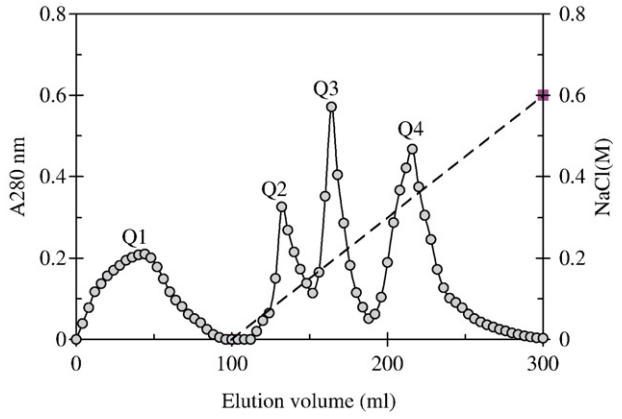

Fig. 1. Anion exchange chromatography of fraction of fruiting body extract, unadsorbed on DEAE-cellulose and subsequently adsorbed on CM-Sepharose and desorbed with buffer containing 0.5 M NaCl, on a Q-Sepharose column (1.0×15 cm). The unadsorbed fraction Q1 was eluted with 0.01 M NH_4HCO_3 buffer (pH 8.6) whereas fractions Q2 and Q3 were desorbed, using a linear NaCl concentration gradient (0 to 0.6 M) in the 0.01 M NH_4HCO_3 buffer (pH 8.5) as indicated by the slanting line across the chromatogram. Fractions Q2 and Q3 were eluted with 0.1 M NaCl and 0.2 M NaCl in 0.01 M NH4HCO3 buffer (pH 8.6), respectively. Hemagglutinating activity was confined to fraction Q3.

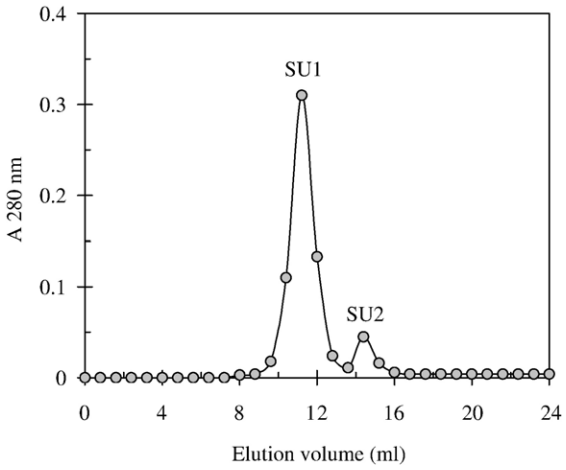

Fig. 2. Gel filtration of fraction Q3 (from Q-Sepharose column) on a Superdex G-75 HR10/30 column, which was eluted with 0.01 M phosphate buffer (pH 7.5) containing 0.15 M NaCl. The flow rate was 0.5 ml/min. Hemagglutinating activity was located in fraction SU1, which had a molecular mass of 32.4 kDa.

thymidine (0.25 μCi, Amersham Biosciences) was added, and the splenocytes were incubated for another 6 h under the same conditions. The splenocytes were then harvested with an automated cell harvester onto a glass fiber filter, and the radioactivity was measured with a Beckman model LS 6000SC scintillation counter. All reported values are the means of triplicate samples.

3. Results

When the saline extract of *P. citrinopileatus* fruiting bodies was passed through a DEAE-cellulose column, the unadsorbed fraction (D1),but not the three adsorbed fractions (D2, D3, D4), exhibited hemagglutinating activity. D1 was subjected to ion exchange chromatography on a CM-cellulose column. After an unadsorbed fraction C1 had been eluted with the starting buffer, the column was eluted stepwise with 0.05 M NaCl, 0.15 M mM NaCl, and 1 M NaCl in the starting buffer. The first adsorbed fraction C2 eluted with 0.05 M NaCl was the peak with hemagglutinating activity. It was chromatographed on Q-Sepharose with a linear gradient of 0–0.2 M NaCl in the same buffer (Fig. 1). Q3, the only active fraction (Table 1), appeared as a major peak (MW 32.4 kDa) upon FPLC-gel filtration on Superdex 75 (Fig. 2). It constituted highly purified lectin (Table 1) which yielded a single band with a molecular mass of 32.4 kDa in SDS-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions (Fig. 3). The N-terminal sequence of the lectin was QYSQMAQVME. A BLAST search did not reveal similarity to any previously reported lectin.

The yields and specific hemagglutinating activities of the various chromatographic fractions are given in Table 1. The purified lectin, with a high hemagglutinating activity, was purified to more than 110 fold. There was about 20% recovery of lectin. The hemagglutinating activity of the lectin remained stable between 20 °C and 60 °C. At 70 °C, 75% of the activity

Table 1
Summary of purification of *P. citrinopileatus* lectin (from 150 g fresh fruiting bodies)

Chromatographic fraction	Yield (mg)	Specific hemagglutinating activity (titer/mg)	Total activity (titer/10^5)	Recovery (%)	Purification fold
Ammonium sulfate precipitate	3152	320	10.01	100	1
D1	419	1792	7.51	75.0	5.6
D2	92	–	–	–	
D3	152	–	–	–	
D4	1620	–	–	–	
C1	170	–	–	–	
C2	25.6	23300	5.96	59.5	73
C3	13.5	–	–	–	
C4	15.2	–	–	–	
Q1	4.5	–	–	–	
Q2	2.9	–	–	–	
Q3	8.9	32790	2.92	29.2	102
Q4	5.1	–	–	–	
SU1	5.7	36437	2.08	20.8	114
SU2	0.7	–	–	–	

– : no activity.

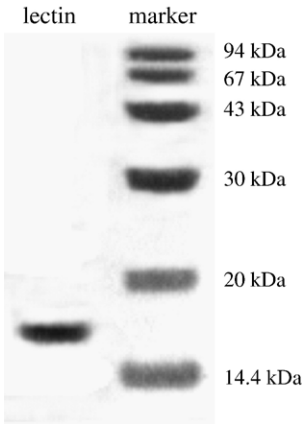

Fig. 3. SDS-PAGE results. The molecular mass of a lectin subunit was 16.2 kDa. The molecular mass of the homodimeric *Pleurotus citrinopileatus* lectin was 32.4 kDa. The proteins used for molecular mass calibration were from Amersham Biosciences and included phosphorylase b (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20 kDa) and α-lactalbumin (14.4 kDa).

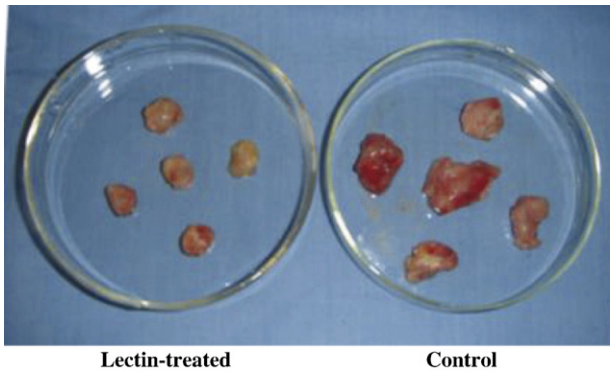

Fig. 4. Sarcomas from control (plate on the right) and lectin-treated (plate on the left) mice. Subcutaneous inoculation of 1×10^5 sarcoma 180 cells in both groups. Lectin-treated group received in addition a daily intraperitoneal injection of 5 mg *Pleurotus citrinopileatus* lectin/kg body weight for 20 consecutive days. The tumor weight in the control group was 2.14±0.23 g and that in the lectin-treated group was 0.46±0.06g (mean±SD, $n=5$, $p<0.01$ compared with control by Students' t test).

was destroyed. The activity of the lectin was further reduced to 12.5% at 80 °C and was completely abolished at 90 °C. The hemagglutinating activity was stable in 0.006 M to 0.1 M NaOH, but was completely abolished in 0.2 M NaOH. Full activity was observed in 0.006 M HCl, but 50% of the activity was left in 0.0125 M to 0.05 M HCl. Only 25% was left in 0.1 M HCl, and no activity was detected in 0.2 M HCl. The majority of divalent and trivalent metallic chlorides tested ($FeCl_3$, $MgCl_2$, $MnCl_2$, $ZnCl_2$ and $CaCl_2$) did not affect the hemagglutinating activity. But the activity of the lectin was reduced to 50% by $PbCl_2$ at a concentration of 0.0125 to 0.02 M. $HgCl_2$ dose-dependently inhibited the activity (75% reduction at 0.00125 M, 87.5% reduction at 0.0025 M, and 100% reduction at and above 0.005 M). The hemagglutinating activity was doubled in the presence of 0.0025 and 0.005 M $AlCl_3$. All sugars tested except inulin,*O*-nitrophenyl-β-D-galactopyranoside and maltose were unable to inhibit the hemagglutinating activity of the lectin when tested at a concentration of 0.2 M. Inulin (0.2 M), *O*-nitrophenyl-β-D-galactopyranoside (0.2 M), O/P-nitrophenyl-β-D-glucuronide, and maltose (0.1 M) exerted an inhibitory effect (Table 2). The lectin inhibited the growth of sarcoma 180 (Fig. 4). It stimulated [methyl-^{3}H] thymidine incorporation into mouse spleen cells *in vitro* (Fig. 5). At 0.12 μM, 0.6 μM and 3 μM, the lectin inhibited HIV-1 reverse transcriptase by 10%, 43.7% and 90.3% respectively. The IC_{50} was 0.93 μM.

Table 2
Effects of various carbohydrates on hemagglutination induced by *P. citrinopileatus* lectin

	Minimum inhibitory concentration of sugar
Inositol	No
Sorbose	No
Raffinose	No
L(+)-Rhamnose	No
D(+)-Mannose	No
Cellobiose	No
D(−)Fructose	No
L(+)-Arabinose	No
Dulcitol	No
D(+)-Xylose	No
D-Melibiose	No
Lactose	No
Inulin	0.1 M
O-Nitrophenyl-β-D-galactopyranoside	0.2 M
Maltose	0.1 M
α-Methyl-D-glucopyranoside	No
O/P-nitrophenyl-β-D-glucopyranoside	No
O/P-nitrophenyl-β-D-glucuronide	0.0125 μM

Note. +, hemagglutination; −, no hemagglutination.
Initial hemagglutinating activity of the lectin was 32 hemagglutinating units. Similar results were obtained when 8 instead of 32 hemagglutinating units were used.
No=no inhibition of hemagglutination when the sugar concentration was 0.2 M.

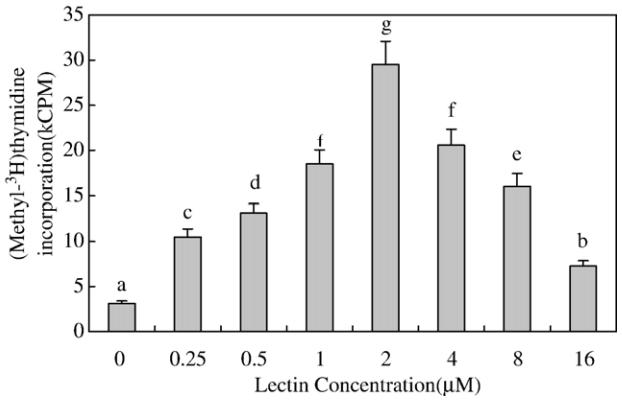

Fig. 5. Mitogenic response of mouse splenocytes to the isolated *P. citrinopileatus* lectin as reflected by uptake of [methyl-^{3}H] thymidine. Different letters (e.g. a for 0 μM and c for 0.25 μM) represent statistically significant ($p<0.05$) difference when the data were analyzed by one-way analysis of variance followed by Duncan's multiple range test.

Table 3
Comparison of characteristics of lectins from *P. citrinopileatus* and *P. ostreatus*

	P. citrinopileatus lectin	*P. ostreatus* lectin [a]	*P. tuber-regium* lectin [b]	*P. cornucopiae mycelial* lectin [c, d]	*P. cornucopiae* fruiting body lectin [e, f]
N-terminal sequence	QYSQMAQVME	ATAKIKATPAQPQQFQPAALN (41-kDa subunit)	DRXAGYVLYXXVPY	TNPTCKGKMVPSAFEPAF	MSYTIKVRVFQTNPNAP
Molecular mass (kDa)	32.4	81	32	40	31
Specific hemagglutinating activity (μ/mg)	36347	30449	7100	19800	–
Sugar specificity	Maltose, *O*-nitrophenyl-β-D-galactopyranoside, inulin	Melibiose, lactose, D-galactose, N-acetylneuraminic acid, raffinose, α-methyl-D-galactopyranoside, inulin	N-acetylglucosamine	N-acetylgalactosamine	–
Thermal stability	Up to 60 °C	Up to 30 °C	ND	ND	ND
Cation activation	Al^{3+}	Al^{3+}, Zn^{2+}	ND	ND	ND
Stability in acid/alkali	No activity in 0.1 M NaOH and 0.1M HCl	100% activity in 0.1 M NaOH, 25% activity in 0.1 M HCl	ND	ND	ND

[a] Data from [8].
[b] Data from [9].
[c] Data from [15].
[d] Data from [13].
[e] Data from [43].
[f] Data from [16].

Table 3 presents a comparison between *P. citrinopileatus* lectin and lectins from other *Pleurotus* species. Their N-terminal sequences, molecular masses, sugar specificity, thermal stability, and acid/alkali stability showed some differences.

4. Discussion

More than 100-fold purification was achieved in the present study on the purification of *P. citrinopileatus* lectin. Four chromatographic steps including three ion exchange steps and one gel filtration step were involved in the purification protocol. The lectin was unadsorbed on DEAE-cellulose, but adsorbed on CM-cellulose and Q-Sepharose. Compared with many other lectins, *P. citrinopileatus* lectin is outstanding in that it has very high hemagglutinating, (36,437 units/mg), HIV-1 reverse transcriptase inhibitory (IC_{50}=0.93 μM), antitumor (about 80% reduction in tumor size) and mitogenic (maximum stimulation at 2 μM) activities. Lectins from the mushroom *Agrocybe cylindracea* [10] and *Agaricus bisporus* [42] exhibit lower hemagglutinating (23915 units/mg) and HIV-1 reverse transcriptase inhibitory (IC_{50}=>8 μM) activities, respectively. The antitumor and mitogenic activities of *P. citrinopileatus* lectin are similar to those of *Pleurotus ostreatus* lectin (88.46% reduction in tumor size) [8] and *A. cylindracea* lectin (maximum stimulation at 2 μM) [10], respectively. It remains to be seen what application *P. citrinopileatus* lectin can find in the therapy of cancer, AIDS and immunoinsufficiency. To date very few lectins have been reported with antifungal activity [41]. *P. citrinopileatus* is devoid of antifungal activity.

P. citrinopileatus lectin is dimeric, like lectins from mushrooms including *A. cylindracea* [10]. Its molecular mass is within the range shown by mushroom lectins [11]. *P. citrinopileatus* lectin possesses a novel N-terminal sequence. It is also unique in that O/P-nitrophenyl-β-D-glucuronide, *O*-nitrophenyl-β-D-galactopyranoside, inulin and maltose are able to inhibit its hemagglutinating activity. Thus immobilized *P. citrinopileatus* lectin can be used as an affinity chromatography media for interaction with molecules containing these sugars. Inclusion of Al^{3+} in buffer would increase the binding activity of the lectin. The sugar binding specificity of the lectin is somewhat surprising. Maltose is a glucose dimer while insulin is a fructose polymer. Both of them are, but neither fructose nor methyl-α-D-glucopyranoside is inhibitory. Furthermore, glucose is classified under Makela's group 3 sugars where C-4 OH is equatorial and galactose is under group 2 sugars where C-4 OH is axial. Normally the hemagglutinating activities of lectins inhibitable by group 2 sugars are not affected by group 3 sugars and vice versa. However, both maltose and *O*-nitrophenyl-β-D-galactopyranoside are inhibitory to *P. citrinopileatus* lectin. *P. citrinopileatus* lectin is stable in high pH but unstable in low pH. It is moderately thermostable, unaffected up to 60 °C. At 70 °C, 25% of the hemagglutinating activity remains. The lack of remarkable pH stability and thermostability reduces the likelihood that these features can find application. The hemagglutinating activity of *P. citrinopileatus* lectin is unaffected in the presence of divalent cations including Ca^{2+}, Zn^{2+}, Mg^{2+}, and $Mn^{2+}$and the trivalent Fe^{3+}, but is curtailed by divalent cations including Hg^{2+} and Pb^{2+} which inactivate many proteins. Irreversible inhibition of *P. citrinopileatus* lectin by Pb^{2+} and Hg^{2+} is probably due to reaction of these heavy-metal ions with the sulfhydryl groups of the lectin to form mercaptides. The hemagglutinating activity of *P. citrinopileatus* lectin is drastically potentiated by the trivalent aluminium ion. A similar phenomenon has been reported for lectin from *P. ostreatus* [8].

This suggests that whereas the divalent cations Ca^{2+}, Mg^{2+}, and Mn^{2+} facilitate the binding of Con A to glycoproteins, Al^{3+} ions and not the other divalent and trivalent cations tested are important to the binding of *P. citrinopileatus* lectin to glycoproteins. However, *P. ostreatus* lectin [8] is less thermostable and acid/alkali stable than *P. citrinopileatus* lectin.

In summary, *P. citrinopileatus* lectin is a novel lectin with potent and potentially exploitable activities. Future research work may include crystallographic studies like that described in [18] for *Agrocybe aegerita* lectin.

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