Hindawi Publishing Corporation
Journal of Biomedicine and Biotechnology
Volume 2010, Article ID 716515, 9 pages
doi:10.1155/2010/716515

Research Article

A Novel Lectin with Antiproliferative and HIV-1 Reverse Transcriptase Inhibitory Activities from Dried Fruiting Bodies of the Monkey Head Mushroom *Hericium erinaceum*

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Received 4 March 2010; Revised 14 April 2010; Accepted 14 April 2010

Academic Editor: Stavros J. Hamodrakas

A lectin designated as *Hericium erinaceum* agglutinin (HEA) was isolated from dried fruiting bodies of the mushroom *Hericium erinaceum* with a chromatographic procedure which entailed DEAE-cellulose, CM-cellulose, Q-Sepharose, and FPLC Superdex 75. Its molecular mass was estimated to be 51 kDa and its N-terminal amino acid sequences was distinctly different from those of other isolated mushroom lectins. The hemagglutinating activity of HEA was inhibited at the minimum concentration of 12.5 mM by inulin. The lectin was stable at pH 1.9–12.1 and at temperatures up to 70°C, but was inhibited by Hg^{2+}, Cu^{2+}, and Fe^{3+} ions. The lectin exhibited potent mitogenic activity toward mouse splenocytes, and demonstrated antiproliferative activity toward hepatoma (HepG2) and breast cancer (MCF7) cells with an IC_{50} of 56.1 μM and 76.5 μM, respectively. It manifested HIV-1 reverse transcriptase inhibitory activity with an IC_{50} of 31.7 μM. The lectin exhibited potent mitogenic activity toward murine splenocytes but was devoid of antifungal activity.

1. Introduction

Lectins are proteins of nonimmune origin which are able to agglutinate cells through sugar-specific binding sites and precipitate polysaccharides and glycoconjugates. Lectins possess at least one non-catalytic domain, which binds reversibly to a specific mono- or oligo-saccharide. Lectins, proteins characterized by their ability to interact with carbohydrates, can be divided into different groups according to their sugar binding specificity [1]. Lectins are abundant in vegetables, fruits, beans and mushrooms [2–6]. *Agaricus bisporus* lectin (ABL) is well documented because it is the most popular edible mushroom in western countries [4, 5]. Other lectins have been found in higher mushrooms, *Sclerotium rolfsii* [7], *Volvariella volvacea* [8], *Ganoderma capense* [9], *Tricholoma mongolicum*, *Pleurotus ostreatus, Agrocybe cylindracea* [10–12], *Schizophyllum commune* [13], *Armillaria luteo-virens* [14], *Agaricus blazei*, *Grifola frondosa*, *Pholiota aurivella*, *Hericium erinaceum*, *Ganoderma lucidum*, *Pleurotus ostreatus*, and *Mycoleptodonoides aitchisonii* [15–21]. Mushroom lectins have different biological activities. Previous studies have demonstrated exploitable biological activities such as antitumor [11], immunomodulatory [8], HIV-1 reverse transcriptase inhibiting [22], cell growth regulating [12], macrophage and lymphocyte activating [23], antiproliferative activities [12], and so on.

Hericium erinaceum, also called monkey head mushroom, is a well known edible and medicinal mushroom in oriental countries. In Japan it is called yamabushitake, and in China it is called houtou which means monkey head [24]. In recent years, *H. erinaceum* has attracted a great deal of attention of owing to its antimicrobial [25], antitumor [26, 27], immunomodulatory [28], antioxidant [29], and cytotoxic activities [27]. Furthermore, it promoties the synthesis of neurogrowth factor [30–33].

A polysaccharide with antitumor activity [34], and a laccase [35] have been reported from *H. erinaceum*. From the fresh fruiting bodies of *H. erinaceum*, a sialic acid-binding

lectin (HEL) with a blocked N-terminal and a molecular mass of approximately 54 kDa has been isolated [17]. The cultures or extracts of *H. erinaceum* processed into tablets have been put into production on a large scale, mainly for curing gastric ulcer and chronic gastricism [36].

In the present study, we isolated and characterized a novel lectin from the dried fruiting bodies of *H. erinaceum*. It possessed a relatively high heat stability and pH stability, a unique amino acid N-terminal amino acid sequence, potent mitogenic activity toward murine spleen cells, reverse transcriptase HIV-1 inhibiting activity and antipoliferative activity on tumor cells. In particular, this lectin is different from HEL in some aspects. Since *H. erinaceum* is used in traditional Chinese medicine, the results of the present study would provide a scientific basis for the medicinal use of this mushroom.

2. Materials and Methods

2.1. Purification Scheme. Dried fruiting bodies (20 g) of the *H. erinaceum* were homogenized in 150 mM NaCl (25 ml/g) using a Waring blender and then soaked in 500 ml of 150 mM NaCl for 12 hours. The slurry was then centrifuged at 8000 × g for 15 minutes. Afterward $(NH_4)_2SO_4$was added into the supernatant to 80% saturation. The precipitate was collected by centrifugation (8000 × g, 4°C, 15 minutes), and dissolved in a small amount of distilled water and dialyzed until the final concentration of 10 mM phosphate-buffered saline (pH 7.0) was attained. The crude extract was then applied to a column of DEAE-cellulose column (Sigma, 1.0 cm × 15 cm) which had been previously equilibrated with 10 mM phosphate-buffered saline (pH 7.0). Following elution of the unadsorbed fraction D1 with the starting buffer, fractions D2 and D3 were obtained by eluting the column with 50 Mm NaCl and 300 mM NaCl in the phosphate-buffered saline respectively. The active fraction (D3) was applied to a CM-cellulose column (Sigma, 1.0 cm × 15 cm) in 10 mM NH_4OAc buffer (pH 5.1). After removal of the unadsorbed protein with the starting buffer, the column was eluted with 50 mM NaCl in the starting buffer to attain the active fraction C2. Subsequently it was further fractionated on an ion exchange chromatography Q-Sepharose column (Sigma, 0.5 × 10 cm). After the unadsorbed fraction (Q1) had been eluted in 10 mM NH_4OAc buffer (pH 5.1), the adsorbed fractions were eluted with a linear gradient of 0–400 mM NaCl in the same buffer. The active peak (Q3) was subjected to final purification on a Superdex G-75 HR 10/30 column by fast protein liquid chromatography using an AKTA Purifier (GE Healthcare, US) and was eluted with 10 mM phosphate buffer (pH 7.5) containing 150 mM NaCl. Peak SU1 represented the purified lectin (HEA).

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 following with the procedure of Laemmli and Favre (1973) [37]. Gel filtration on a performed Superdex 75 HR 10/30 column (GE Healthcare, US), which had been calibrated with molecular mass markers, was also obtained for the molecular mass determination of the lectin. The N-terminal sequence of the lectin was obtained by using a Hewlett-Packard HP G1000A Edman degradation unit and an HP 1000 HPLC System [37].

2.3. Assay of Lectin (Hemagglutinating) Activity. In the assay for lectin (hemagglutinating) activity, a serial two-fold delution of the lectin solution in microtiter U-plates (50 μl) was mixed with 50 μl of 2% suspension of rabbit red cells in phosphate-buffered saline (pH 7.2) at 20°C. The results were recorded after about 1 hour when the blank had fully sedimented. The hemagglutintion titer, defined as the reciprocal of the highest dilution of the samples exhibiting hemagglutination, was reckoned as one hemagglutination unit. Specific activity is the number of hemagglutination units per mg protein [11].

The hemagglutination inhibition tests to investigate inhibition of lectin-induced hemagglutination by various carbohydrates were executed in a way similar to the hemagglutination test. The carbohydrates examined comprised inulin, D-melibiose, D-fructose, L-arabinose, L-rhamnose, D-xylose, L-sorbose, inositol, lactose, D-galactose, sorbose, D-arabinose, adonitol, cellobiose, D-glucose, D-mannose, raffinose, turanose, sucrose, maltose, and dulcitol. 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 permitted to stand for 30 minutes 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 computed [11].

The effects of NaOH, HCl, metal chlorides, and temperature on hemagglutininating activity of the lectin were studied as previously described in [11, 12].

2.4. Assay of Antiproliferative Activity on Tumor Cell Lines. The tumor cell lines, human breast cancer (MCF7) and hepatoma (HepG2), were acquired from American Type Culture Collection (ATCC). They were kept in Dulbecco modified Eagl's medium (DMEM) containing 10% fetal bovine serum (FBS), 100 mg/L streptomycin and 100 IU/ml penicillin at 37°C in a humidified atmosphere of 5% CO_2. Cells (1×10^4) in their exponential growth phase were seeded into each well of a 96-well culture plate (Nunc, Denmark). Incubation was carried out for 3 hours before addition of the lectin. Incubation was then continued for another 48 hours. Radioactive precursor, 1 μCi, ([^{3}H-methyl] thymidine, from GE Healthcare) was then added to each well and incubated for 6 hours. The cultures were then harvested by means of a cell harvester. The incorporated radioactivity was determined by liquid scintillation counting [44].

2.5. Assay for HIV-1 Reverse Transcriptase Inhibitoryactivity. The inhibitory activity towards human immunodeficiency

Table 1: Summary of purification of HEA (from 20 g dried fruit bodies).

Chromatographic fraction	Yield (mg/20 g)	Total Hemagglutinating Activity $U \times 10^5$	Specific hemagglutinating Activity (U/mg)	Recovery hemagglutinating Activity (%)
Crude Extract	**11840**	**3.6**	**30.64**	**100**
Ammonium sulfate precipitate	**989.6**	**5.12**	**517**	**8.358**
D1	149.71	—	—	—
D2	67.5	—	—	—
D3	**175.058**	**1.66**	**950**	**1.4**
D4	759.75	1.6	2.1	6.4
C1	50.754	—	—	—
C2	**27.516**	**0.544**	**1978**	**0.23**
C3	45.528	—	—	—
C4	20.985	—	—	—
Q1	0.6044	—	—	—
Q2	8.3924	—	—	—
Q3	**13.34**	**0.512**	**3838**	**0.11**
SU1	**10.35**	**0.5**	**4830**	**0.087**
SU2	3.04	—	—	—

The lectin-enriched fractions and the associated data are highlighted in boldface.

virus type 1 (HIV-1) reverse transcriptase (RT) was evaluated by using an enzyme-linked immunosorbent assay (ELISA) kit from Boehringer Mannheim (Germany). The assay makes use of the ability of reverse transcriptase to synthesize DNA, commencing from the template/primer hybrid poly(A)-oligo$(dT)_{15}$. The digoxigenin- and biotin-labeled nucleotides in an optimized ratio are incorporated into the DNA molecule synthesized by the RT. The detection and quantification of the synthesized DNA as a measure of RT activity follows sandwich ELISA protocol. A fixed amount (4–6 ng) of recombinant HIV-1 RT was used. The inhibitory activity of the lectin was expressed as percent inhibition as compared to a control without the protein [45].

2.6. Assay of Mitogenic Activity Toward Mouse Splenocytes. Four C57BL/6 mice (20–25 g) were sacrificed by cervical dislocation and the spleens were aseptically excised. Splenocytes were isolated by pressing the tissue through a sterilized 100-mesh stainless steel sieve and respended to 5×10^6 cells/ml in RPMI 1640 culture medium containing 10% fetal bovine serum, 100 units penicillin/ml, and 100 μg streptomycin/ml. The splenocytes (7×10^5 cells/0.1 ml/well) were seeded into a 96-well culture microplates and a serial dilution of the lectin in 100 μl medium was added. Following incubation of the splenocytes at 37°C in a humidified atmosphere of 5% carbon dioxide in the presence or absence of the lectin for 24 hours, the cells in one well were pulsed with 10 μCi [^{3}H-methyl] thymidine (specific activity 0.25 μCi/mmol, GE Healthcare) was added, and the splenocytes were incubated for another 6 hours under the same conditions, and then harvested onto a glass fiber filter using an automated cell harvester. The radioactivity was counted using a Beckman model LS 6000SC scintillation counter. The proliferative (mitogenic) response was expressed as mean counts per minute (cpm) [44]. All reported values are the means of triplicate samples.

2.7. Assay of Antifungal Activity. The assays were conducted as detailed by Wang and Ng [46]. The assay for inhibitory activity toward the fungi *Fusarium oxysporum*, *Rhizoctonia cerealis*, *Rhizoctonia solani*, and *Sclerotinia sclerotiorum* was carried out in 100 mM × 15 mM petri dishes containing 10 ml of potato dextrose agar (PDA). After formation of the mycelial colony, sterile blank paper disks (0.625 cm in diameter) were deposited at a distance of 0.5 cm away from the circumference of the mycelial colony. An aliquot (15 μl) of the lectin was added to a disk. The dishes were incubated at 25°C for 72 hours until mycelial growth had encircled the disks containing the control and had produced crescents of inhibition around disks containing samples with antifungal activity.

3. Results and Discussion

3.1. Isolation and Purification of HEA. Hemagglitinating activity in the fruiting body extract was adsorbed successively on DEAE-cellulose, CM-cellulose, and Q-Sepharose columns. Activity was located in peaks D3, C2, and Q3 (Figures 1(a), 1(b) and 1(c)). The purified fraction SU1 was obtained through gel filtration on a Superdex G-75 HR 10/30 column (Figure 1(d)). The yields and specific hemagglutinating activities of the various chromatographic

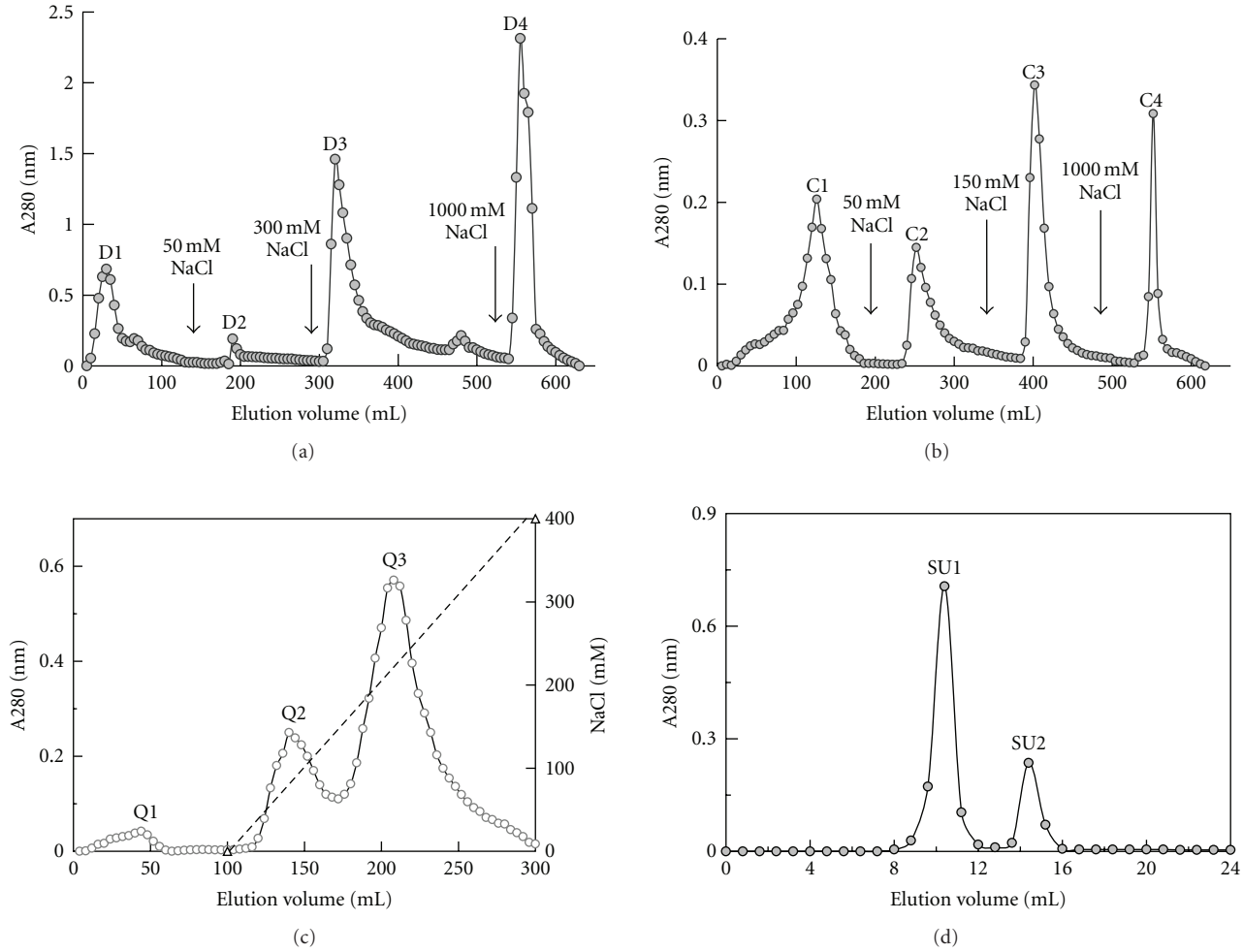

Figure 1: Elution profiles of HEA. (a) Anion exchange chromatography of protein derived from the $(NH_4)_2SO_4$ precipitate of crude extract of *H. erinaceum* fruiting bodies on a DEAE-cellulose column (1.0 cm × 20 cm). which was equilibrated and eluted with 10 mM phosphate-buffered (pH 7.0). Arrows indicate elution of the column stepwise with 50 mM, 300 mM and 1000 mM NaCl in the starting buffer. (b) Ion exchange chromatography of D3 on a CM-cellulose column (2.0 cm × 15 cm). Arrows indicate elution of the column stepwise with 50 mM, 150 mM and 1000 mM NaCl in 10 mM NH_4OAc buffer (pH 5.1). (c) Ion exchange chromatography of fraction C2 on a Q-Sepharose column (1.0 cm × 10 cm). The slanting line across the chromatogram denotes a linear NaCl concentration (0 to 400 mM) gradient in 10 mM NH_4OAc buffer (pH 5.1). (d) Gel filtration of fraction Q3 from Q-Sepharose column on a Superdex G-75 column, which was eluted with 10 mM phosphate buffer (pH 7.5) containing 150 mM NaCl. The flow rate was 0.4 ml/min.

fractions are given in Table 1. The purified lectin appeared as a single band with a molecular mass of 51 kDa in SDS/PAGE (Figure 2) and a peak (SU1) with a molecular mass of 51 kDa in FPLC gel filtration. The N-terminal sequence of the HEA was AFGQLSFANLAAADF, different from the other mushroom lectins shown in Table 2. A blast search revealed that there was only slight resemblance to other previously reported lectins.

HEA isolated in the present investigation differed from lectins purified from other mushrooms. HEA was adsorbed on DEAE-cellulose and CM-cellulose, and eluted with 300 mM NaCl and 50 mM NaCl, respectively. It was deadsorbed from Q-Sepharose column with a linear NaCl concentration gradient (0 to 400 mM). Compared with HEA, another *H. erinaceum* lectin HEL was adsorbed DEAE-Toyopearl column and Mono-S column. HEL could be also purified from the ammonium sulfate precipitate by affinity chromatography on BSM- or asialo-BSM-Toyopearl. But, recovery of the activity by affinity chromatography was much lower than that of this study procedure (10% and 8.7%, resp.) [17]. What is more, in each step a substantial amount of protein devoid of hemagglutininating activity was eliminated, indicating that the procedure was an effective one. Different from HEL which is composed of two different subunits with a molecular mass of 15 kDa and 16 kDa, HEA is monomeric with a molecular mass approximating 51 kDa. HEA displayed an N-terminal sequence with little resemblance to some of the published mushroom lectins, such as those from *Agrocybe aegerita*, *Laccaria bicolor*, *Coprinopsis cinerea*, *Thermophilanous ganoderma*, *Agaricus bisporus*, *Pleurotus cornucopiae*, *Flammulina velutipes*, and *Grifola frondosa* (shown in Table 2) [9, 18, 38–43].

Table 2: N-terminal sequence of HEA with other mushroom lectins.

Species	N-terminal sequence	Reference number
Hericium erinaceum	AFGQ**L**S**FANLAA**A**D**F	this study
Laccaria bicolor	SH**L**YGDGV**A**L	[38]
Coprinopsis cinerea	IPLEGT**F**G **D**R	[39]
Thermophilanous ganoderma	VNDYE**AN**YG**A**D**D**	[9]
Pleurotus cornucopiae	SDSTWT**FA**M**L**	[40]
Agaricus bisporus	MGGSGTSGS**L**	[41]
Agrocybe aegerita	NISAGTSVD**L**	[42]
Flammulina velutipes	TSLTFQLAY**L**	[43]
Grifola frondosa	NWPAEMMID**L**KHPIVEMR	[18]

Identical amino acid residues are highlighted in boldface and underscored.

Table 3: Effect of temperature on hemagglutinating activity of HEA.

Temperature (°C)	20	30	40	50	60	70	80	90	100
Hemagglutinating activity (U)	64	64	64	64	64	64	32	0	0

Initial hemagglutinating activity of HEA solution was 64 hemagglutinating units.

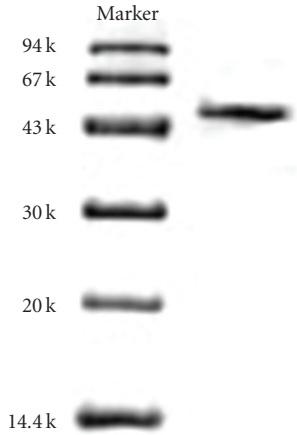

Figure 2: SDS-PAGE of fraction SU1 (purified HEA in the right line) from Superdex 75 column. Molecular mass markers (left lane) are: phosphorylase b (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20 kDa) and α-lactalbumin (14.4 kDa).

3.2. Biological Characteristics of HEA. The specificities of carbohydrate-binding of HEA were examined by hemagglutination-inhibition assay. The hemagglutinating activity of HEA (32 units) was totally inhibited by inulin at a concentration of 12.5 mM (data not shown). Carbohydrate specificity was an important characteristic of lectins. It is interesting that only inulin, a plant polysaccharide, was able to inhibit the hemagglutinating activity of HEA. To date several inulin-specific lectins have been reported [14, 47, 48]. Hence HEA may be used in the production of immobilized lectin for affinity chromatography.

The hemagglutinating activity of HEA remained stable between 20°C and 70°C. The activity was curtailed to half at 80°C, and was completely abolished at 90°C (Table 3).

Table 4: Effects of NaOH and HCl solutions on hemagglutinating activity of HEA.

pH value	Hemagglutinating activity remaining (%)	pH value	Hemagglutinating activity remaining (%)
0.7	0	11.8	100
1.0	0	12.1	100
1.3	0	12.4	25
1.6	12.5	12.7	25
1.9	100	13.0	12.5
2.1	100	13.3	0

Initial hemagglutinating activity of HEA solution was 32 hemagglutinating units.

Mushroom lectins differ from one another in thermostability. The lectin from *G. capense* was not attenuated following treatment at 100°C for 60 minutes [9], while the hemagglutinating activity of *P. ostreatus* lectin was reduced at and above 40°C [11]. However, the hemagglutinating activity of HEA was stable in the pH solutions range 1.9–12.1. When the pH value reached 1.3 or 13.3, the hemagglutinating activity was completely eliminated (Table 4). HEA exhibited some similarity to the lectin from *Armillaria luteo-virens* in pH stability [14]. Compared with some lectins that are unstability to low, and high pH [13, 44], HEA was much more robust in that it was not adversely affected by changes in the ambient pH.

The majority of univalent, divalent and trivalent metallic chlorides including K^+, Fe^{2+}, Mn^{2+}, Zn^{2+}, Pb^{2+}, Co^{2+}, Mg^{2+}, Ca^{2+} and Al^{3+} ions, did not affect the lectin activity. But the activity was inhibited by Cu^{2+} and Hg^{2+} ions at a concentration of 5 mM and 10 mM, respectively. The hemagglutinating activity was completely suppressed by Fe^{3+} ions at a concentration of 12.5 mM (Table 5). In the

Table 5: Effects of cations on hemagglutinating activity of HEA.

	1.25 mM	2.5 mM	5 mM	10 mM	20 mM
K^+	32	32	32	32	32
Fe^{2+}	32	32	32	32	32
Zn^{2+}	32	32	32	32	32
Ca^{2+}	32	32	32	32	32
Mg^{2+}	32	32	32	32	32
Mn^{2+}	32	32	32	32	32
Pb^{2+}	32	32	32	32	32
Al^{3+}	32	32	32	32	32
Co^{2+}	32	32	32	32	32
Cu^{2+}	32	32	0	0	0
Hg^{2+}	32	32	32	0	0
Fe^{3+}	32	0	0	0	0

Initial hemagglutinating activity of HEA solution was 32 hemagglutinating units.

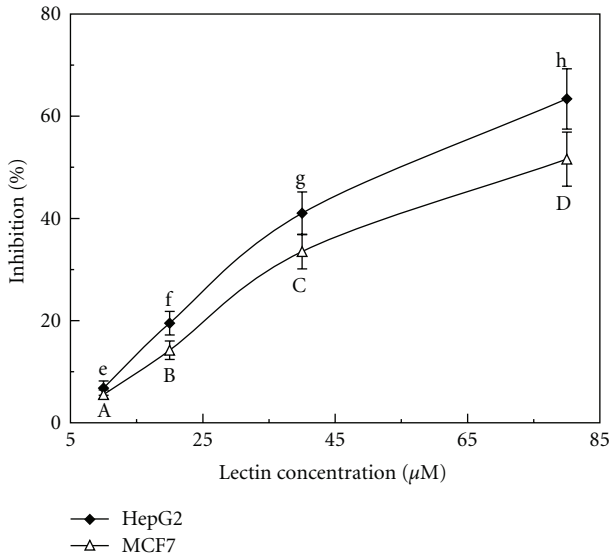

Figure 3: Inhibitory effect of HEA on proliferation of Hep G2 and MCF-7 cancer cells line in vitro. Results represent mean ± SD ($n = 3$). The IC_{50} values toward HepG2 cells and MCF7 cells are 56.1 μM and 76.5 μM, respectively. Different alphabets next to the data points indicate statistically significant difference ($P < .05$) when the data are analyzed by analysis of variance followed by Duncan's multiple range test.

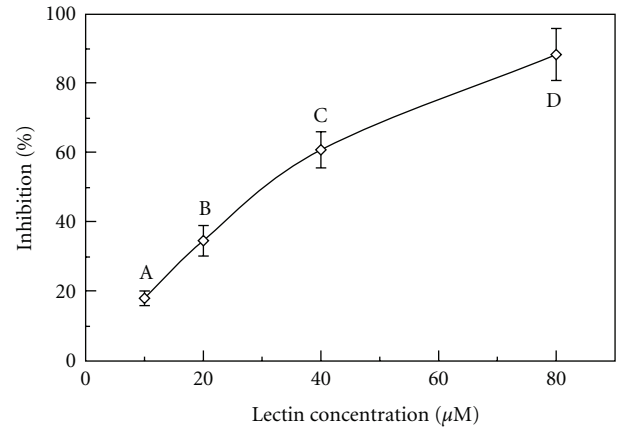

Figure 4: Inhibitory effect of HEA on activity of HIV-1 reverse transcriptase. Results represent mean ± SD ($n = 3$). The IC_{50} value is 31.7 μM. Different alphabets next to the data points indicate statistically significant difference ($P < .05$) when the data are analyzed by analysis of variance followed by Duncan's multiple range test.

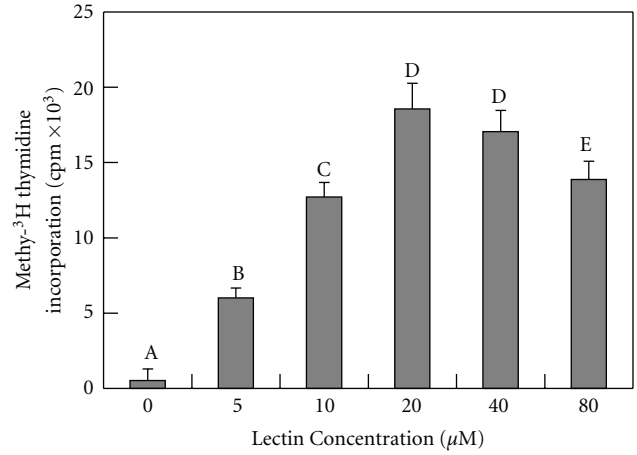

Figure 5: Mitogenic response of murine splenocytes to the lectin isolated from *H. erinaceum* as reflected by uptake of [methyl-^{3}H] thymidine uptake in vitro. Results represent means ± SD ($n = 3$). Maximal stimulation of thymidine uptake was brought about by a lectin concentration of 20 μM. There was an approximately 36-fold increase over the control value without lectin. Different alphabets next to the data points indicate statistically significant difference ($P < .05$) when the data are analyzed by analysis of variance followed by Duncan's multiple range test.

present study, Cu^{2+}, Fe^{3+}, and Hg^{2+} ions when presented at 5 mM, 2.5 mM, and 10 mM, respectively, diminished the hemagglutinating activity of HEA. In contrast, Ca^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, AL^{3+}, Pb^{2+}, K^+, Co^{2+}, and Fe^{2+} ions were devoid of any effect. It is known that different mushroom lectins might be affected differently by the ions. Al^{3+} ions strongly potentiated the hemagglutinating activity of *P. ostreatus* and *P. citrinopileatus* lectin [11, 44], but did not influence the lectin from *S. commune* [13]. Ca^{2+}, Mg^{2+}, Mn^{2+}, and Zn^{2+} ions did not alter the hemagglutinating activity of lectins isolated from *Amanita pantherina* [49], *G. frondosa* [18], and *H. erinaceum* [17], but inhibited *S. commune* lectin [13].

3.3. Other Biological Activities of HEA. Lectins from *A. bisporus*, *P. ostreatus*, *T. mongolicum*, and *V. volvacea* exhibit antitumor activity in vivo or antiproliferative activity in vitro [5, 11, 23]. *A. luteo-virens* was devoid of antiproliferative activity on HepG2 cells [14]. HEA inhibited the proliferation of HepG2 and MCF7 tumor cells with an IC_{50} value of 56.1 μM and 76.5 μM, respectively (Figure 3). The potent antiproliferative activity of HEA is remarkable and hopefully it can be developed into an aided agent for cancer therapy.

To date very few lectins have been reported with antifungal activity and the number of lectins claimed to

Table 6: Comparison of characteristics of HEL [17] and HEA (this study).

Characteristics	HEL	HEA
Chromatographic behavior on		
DEAE ion exchanger	not determined	adsorbed, eluted with 300 mM NaCl
DEAE-Toyopearl column	adosorbed, eluted with 200 mM NaCl in Tris buffer.	not determined
CM ion exchanger	not determined	adsorbed, eluted with 50 Mm NaCl
Mono-S column	Absorbed	not determined
Moleculr Mass (kDa)	54	51
Subunit molecular mass (kDa)	15 and 16	51
N-terminal sequence	not determined	AFGQLSFANLAAADF
Thermostability	Up to 70°C	up to 70°C
pH stability	5.0–10.5	1.9–12.1
Sugar specificity		
(i) mono- or oligo-saccharides	NeuGc, NeuAc, 3′-N-Acetylneuramin-lactose and galacturonic acid	inulin and not by simple sugars
(ii) glycoproteins	asialo-BSM, Asialofetuin	not determined
Sialic acid-binding activating	Yes	not determined
Effect of cations on hemagglutinating activity	no effect	activity inhibited by Cu^{2+}, Fe^{3+}, and Hg^{2+} ions
Antifungal activity	not determined	no effect
Antiproliferative activity	not determined	IC_{50} of 56.1 μM toward HepG2 cells, and 76.5 μM toward MCF7 cells, respectively
Mitogenic activity toward splenocytes	not determined	35.8 fold increase over basal value
HIV-1 reverse transcriptase inhibitory activity	not determined	IC_{50} = 31.7 μM

have antifungal activity is also relatively small [50–53]. The lectin lacked antifungal and ribonuclease activities (data not shown). HIV-RT is a key enzyme of the HIV life cycle. Screening of HIV-RT inhibitors is currently a strategy to search for anti-HIV drugs. It is possible that the mechanism of inhibition is analogous to the protein–protein interaction involved in the inhibition of HIV-1 reverse transcriptase by the homologous protease [54]. It is worth mentioning that HEA manifested a weak potent inhibitory activity toward HIV-1 RT (IC_{50} = 31.7 μM, Figure 4), compared with other lectins such as *S. commune* lectin (IC_{50} = 1.2 μM), *P. citrinopileatus* lectin (IC_{50} = 0.93 μM) and *P. adiposa* lectin (IC_{50} = 1.9 μM), respectively [13, 44, 48]. However, The *P. ostreatus* lectin and *G. capense* lectin lacked any inhibitory effect on HIV-1 reverse transcriptase [9, 11]. It manifested potent mitogenic activity toward murine spleen cells, producing a 35.8-fold stimulation over the control value at at a dose of 20 μM (Figure 5). The mitogenic activity of HEA toward murine splenocytes is in accordance with reports on lectins from the mushrooms *Volvariella volvacea* [8, 55] and A*grocybe cylindracea* [9].

3.4. Comparison with HEA and HEL. A comparison with HEL [17] and HEA (this study) is listed in Table 6. N-terminal sequence and many biological activities, including antifungal activity, antiproliferative activity, mitogenic activity and HIV-1 RT inhibitory activity have been determined in HEA. The N-terminal sequence, antifungal activity, antiproliferative activity and HIV-1 RT inhibitory activity of HEL has not been demonstrated. However, the molecular mass of HEL is different from HEA. HEL has a molecular mass in the vicinity of 54 kDa and composed of two different subunits with a molecular mass of 15 and 16 kDa, while HEA in this study was monomeric and had a molecular mass about 51 kDa. The two lectins have very similar the thermostability. The pH stability of HEA (pH 1.9–12.1) is much higher than that of HEL (pH 5.0–10.5). The hemagglutinating activity of the HEA but not that of HEL was inhibited by Cu^{2+}, Fe^{3+}, and Hg^{2+} ion. HEA and HEL had distinctive sugar specificities. HEL could interact with N-glycolylneuramic acid, N-acetylneuramic acid, 3′-N-acetylneuramin-lactose and galacturonic acid, while HEA was only influenced by inulin.

4. Conclusions

In summary, a novel lectin named HEA was isolated from dried fruiting bodies of the mushroom. It possessed a distinctive N-terminal sequence, carbohydrate specificity, and potent antiproliferative activity toward tumor cell lines, potent mitogen activity toward splenocytes and HIV-1 RT inhibitory activity. It represents an addition to the existing list of mushroom lectins.

Acknowledgments

This work was financially supported by National Grants of China (2007BAD89B00 and 2010CB732202).

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