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

Yanrui Li,1 Guoqing Zhang,1 Tzi Bun Ng,2 and Hexiang Wang1

1 State Key Laboratory for Agrobiotechnology, Department of Microbiology, China Agricultural University, Beijing 100193, China
2 School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong

Correspondence should be addressed to Tzi Bun Ng, b021770@mailserv.cuhk.edu.hk and Hexiang Wang, hxwang1957@gmail.com

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

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*, *Arroyoey cynthiaeae* [10–12], *Schizophyllum commune* [13], *Armillaria luteo-vires* [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 promotes 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 antiproliferative 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, (NH₄)₂SO₄ 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 NH₄OAc 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). The unadsorbed fraction (Q1) had been eluted in 10 mM NH₄OAc 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 twofold dilution 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 hemagglutination 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-xylene, 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 hemagglutinating activity of the lectin were studied as previously described in [11, 12].

#### 2.4. Assay for 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 Eagle’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% CO₂. Cells (1 × 10⁴) 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, ([³H-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 Inhibitory Activity

The inhibitory activity towards human immunodeficiency...
Table 1: Summary of purification of HEA (from 20 g dried fruit bodies).

| Chromatographic fraction | Yield (mg/20g) | Total Hemagglutinating Activity | Specific Hemagglutinating Activity | 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 resuspended to $5 \times 10^6$ cells/ml in RPMI 1640 culture medium containing 10% fetal bovine serum, 100 units penicillin/ml, and 100 μg streptomycin/ml. The splenocytes ($7 \times 10^5$ 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 [3H-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. Hemagglutinating 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
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 desorbed 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 hemagglutinating 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*, *Thermophilous 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             | AFGQLSEANLAAADF     | this study       |
| Laccaria bicolor               | SHLYGDGVAL          | [38]             |
| Coprinopsis cinerea            | IPLEGTFG            | [39]             |
| Thermophilous ganoderma        | VNDYEANYGADD        | [9]              |
| Pleurotus cornucopiae          | SDSTWTFAML          | [40]             |
| Agaricus bisporus              | MGGGTSGSL           | [41]             |
| Agrocybe aegerita              | NISAGTSVDL          | [42]             |
| Flammulina velutipes           | TSLTFQLAY           | [43]             |
| Grifola frondosa               | NWPAEMMIDKHIPIVEMR  | [18]             |

Identical amino acid residues are highlighted in boldface and underscored.

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

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

Initial hemagglutinating activity of HEA solution was 64 hemagglutinating units.

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

| pH value | Hemagglutinating activity remaining (%) | 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.

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). 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⁺, Fe²⁺, Mn²⁺, Zn²⁺, Pb²⁺, Co²⁺, Mg²⁺, Ca²⁺ and Al³⁺ ions, did not affect the lectin activity. But the activity was inhibited by Cu²⁺ and Hg²⁺ ions at a concentration of 5 mM and 10 mM, respectively. The hemagglutinating activity was completely suppressed by Fe³⁺ ions at a concentration of 12.5 mM (Table 5). In the

![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).](image-url)
Table 5: Effects of cations on hemagglutinating activity of HEA.

| Cation   | 1.25 mM | 2.5 mM | 5 mM  | 10 mM | 20 mM |
|----------|---------|--------|-------|-------|-------|
| K⁺       | 32      | 32     | 32    | 32    | 32    |
| Fe²⁺     | 32      | 32     | 32    | 32    | 32    |
| Zn²⁺     | 32      | 32     | 32    | 32    | 32    |
| Ca²⁺     | 32      | 32     | 32    | 32    | 32    |
| Mg²⁺     | 32      | 32     | 32    | 32    | 32    |
| Mn²⁺     | 32      | 32     | 32    | 32    | 32    |
| Pb²⁺     | 32      | 32     | 32    | 32    | 32    |
| Al³⁺     | 32      | 32     | 32    | 32    | 32    |
| Co²⁺     | 32      | 32     | 32    | 32    | 32    |
| Ca²⁺     | 32      | 32     | 32    | 32    | 32    |
| Hg²⁺     | 32      | 32     | 32    | 0     | 0     |
| Cu²⁺     | 32      | 32     | 32    | 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 IC₅₀ 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.

present study, Cu²⁺, Fe³⁺, and Hg²⁺ ions when presented at 5 mM, 2.5 mM, and 10 mM, respectively, diminished the hemagglutinating activity of HEA. In contrast, Ca²⁺, Mg²⁺, Mn²⁺, Zn²⁺, Al³⁺, Pb²⁺, K⁺, and Fe²⁺ ions were devoid of any effect. It is known that different mushroom lectins might be affected differently by the ions. Al³⁺ 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]. Ca²⁺, Mg²⁺, Mn²⁺, and Zn²⁺ 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].

Figure 4: Inhibitory effect of HEA on activity of HIV-1 reverse transcriptase. Results represent mean ± SD (n = 3). The IC₅₀ 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.

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 IC₅₀ 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                                | adsorbed, eluted with 200 mM NaClin Tris buffer. | not determined                   |
| CM ion exchanger                                     | not determined                   | adsorbed, eluted with 50 mM NaCl |
| Mono-S column                                        | Absorbed                         | not determined                   |
| Molar 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-Acetyleneuramin-lactose and galacturonic acid | inulin and not by simple sugars |
| (ii) glycoproteins                                   | asialo-BSM, Asialofetuin         | not determined                   |
| Sialic acid-binding activating                       | Yes                              | activity inhibited by Cu^{2+}, Fe^{3+}, and Hg^{2+} ions |
| Effect of cations on hemagglutinating activity       | no effect                        | no effect                        |
| Antifungal activity                                  | not determined                   | IC_{50} of 56.1 μM toward HepG2 cells, and 76.5 μM toward MCF7 cells, respectively |
| Antiproliferative activity                           | not determined                   | 35.8 fold increase over basal value |
| Mitogenic activity toward splenocytes                | not determined                   |                                  |
| 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 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 Agrocybe cylindracea [9].

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).

References

[1] T. B. Ng, "Peptides and proteins from fungi," Peptides, vol. 25, no. 6, pp. 1055–1073, 2004.
[2] S. Oguri, M. Kamoshida, Y. Nagata, Y. S. Momonoki, and H. Kamiyama, "Characterization and sequence of tomato 2S seed albumins: a storage protein with sequence similarities to the fruit lectin," Planta, vol. 216, no. 6, pp. 976–984, 2003.
[3] W. J. Peumans, W. Zhang, A. Barre, C. Houlès Astoul, P. J. Balint-Kurti, P. Rovira, P. Rougé, G. D. May, F. Van Leuven, P. Truffa-Bachi, and E. J. M. Van Damme, "Fruit-specific lectins from banana and plantain," Planta, vol. 211, no. 4, pp. 546–554, 2000.
[4] W. J. Peumans and E. J. Van Damme, "Lectins as plant defense proteins," Plant Physiology, vol. 109, no. 2, pp. 347–352, 1995.
[5] H. Wang, T. B. Ng, and V. E. C. Ooi, "Lectins from mushrooms," Mycological Research, vol. 102, no. 8, pp. 897–906, 1998.
[6] R. Loris, T. Hamelryck, J. Bouckaert, and L. Wyns, "Legume lectin structure," Biochimica et Biophysica Acta, vol. 1383, no. 1, pp. 9–36, 1998.
[7] A. M. Wu, J. H. Wu, M.-S. Tsai, G. V. Hegde, S. R. Inamdar, B. M. Swamy, and A. Herp, "Carbohydrate specificity of a lectin isolated from the fungus Sclerotium rolfsii," Life Sciences, vol. 69, no. 17, pp. 2039–2050, 2001.
[8] Q.-B. She, T.-B. Ng, and W.-K. Liu, "A novel lectin with potent immunomodulatory activity isolated from both fruiting bodies and cultured mycelia of the edible mushroom Volvariella volvacea," Biochemical and Biophysical Research Communications, vol. 247, no. 1, pp. 106–111, 1998.
[9] P. H. K. Ngai and T. B. Ng, "A mushroom (Ganoderma capense) lectin with spectacular thermostability, potent mitogenic activity on splenocytes, and antiproliferative activity toward tumor cells," Biochemical and Biophysical Research Communications, vol. 314, no. 4, pp. 988–993, 2004.
[10] H. Wang, T. B. Ng, and Q. Liu, "Isolation of a new heterodimeric lectin with mitogenic activity from fruiting bodies of the mushroom Agrocybe cylindracea," Life Sciences, vol. 70, no. 8, pp. 877–885, 2002.
[11] H. Wang, I. Gao, and T. B. Ng, "A new lectin with highly potent antitumor and antiangiogenic activities from the oyster mushroom Pleurotus ostreatus," Biochemical and Biophysical Research Communications, vol. 275, no. 3, pp. 810–816, 2000.
[12] H. X. Wang, T. B. Ng, W. K. Liu, V. E. C. Ooi, and S. T. Chang, "Isolation and characterization of two distinct lectins with antiproliferative activity from the cultured mycelium of the edible mushroom Tricholoma mongolicum," International Journal of Peptide and Protein Research, vol. 46, no. 6, pp. 508–513, 1995.
[13] C. H. Han, Q. H. Liu, T. B. Ng, and H. X. Wang, "A novel homodimeric lactose-binding lectin from the edible split gill medicinal mushroom Schizophyllum commune," Biochemical and Biophysical Research Communications, vol. 336, no. 1, pp. 252–257, 2005.
[14] K. Feng, Q. H. Liu, T. B. Ng, H. Z. Liu, J. Q. Li, G. Chen, H. Y. Sheng, Z. L. Xie, and H. X. Wang, "Isolation and characterization of a novel lectin from the mushroom Armillaria luteo-virens," Biochemical and Biophysical Research Communications, vol. 345, no. 4, pp. 1573–1578, 2006.
[15] H. Kawagishi, Y. Abe, T. Nagata, A. Kimura, and S. Chiba, "A lectin from the mushroom Pholiota aurivella," Agricultural and Biological Chemistry, vol. 55, no. 10, pp. 2485–2489, 1991.
[16] H. Kawagishi, S.-I. Mitsunaga, M. Yamawaki, M. Ido, A. Shimada, T. Kinoshita, T. Murata, T. Usui, A. Kimura, and S. Chiba, "A lectin from mycelia of the fungus Ganoderma lucidum," Phytochemistry, vol. 44, no. 1, pp. 7–10, 1997.
[17] H. Kawagishi, H. Mori, A. Uno, A. Kimura, and S. Chiba, "A sialic acid-binding lectin from the mushroom Hericium erinaceum," FEBS Letters, vol. 340, no. 1–2, pp. 56–58, 1994.
[18] H. Kawagishi, A. Nomura, T. Mizuno, A. Kimura, and S. Chiba, "Isolation and characterization of a lectin from Grifola frondosa fruiting bodies," Biochimica et Biophysica Acta, vol. 1034, no. 3, pp. 247–252, 1990.
[19] H. Kawagishi, A. Nomura, T. Yumen, T. Mizuno, T. Hagiwara, and T. Nakamura, "Isolation and properties of a lectin from the fruiting bodies of Agaricus blazei," Carbohydrate Research, vol. 183, no. 1, pp. 150–154, 1988.
[20] H. Kawagishi, H. Suzuki, H. Watanabe, H. Nakamura, T. Sekiguchi, T. Murata, T. Usui, K. Sugiyama, H. Saganuma, T. Inakuma, K. Ito, Y. Hashimoto, M. Ohnishi-Kameyama, and T. Nagata, "A lectin from an edible mushroom Pleurotus ostreatus as a food intake-suppressing substance," Biochimica et Biophysica Acta, vol. 1474, no. 3, pp. 299–308, 2000.
[21] H. Kawagishi, J.-I. Takagi, T. Taira, T. Murata, and T. Usui, "Purification and characterization of a lectin from the mushroom Mycoelotodonoides aitchisonii," Phytochemistry, vol. 56, no. 1, pp. 53–58, 2001.
[22] H. X. Wang and T. B. Ng, "Examination of lectins, polysaccharopeptide, polysaccharide, alkaloid, coumarin and trypsin inhibitors for inhibitory activity against human immunodeficiency virus reverse transcriptase and glycohydrolases," Planta Medica, vol. 67, no. 7, pp. 669–672, 2001.
[23] H. X. Wang, T. B. Ng, W. K. Liu, V. E. C. Ooi, and S. T. Chang, "Polysaccharide-peptide complexes from the cultured mycelia of the mushroom Coriolus versicolor and their culture medium activate mouse lymphocytes and macrophages," International Journal of Biochemistry and Cell Biology, vol. 28, no. 5, pp. 601–607, 1996.
[24] B.-K. Yang, J.-B. Park, and C.-H. Song, "Hypolipidemic effect of an exo-biopolymer produced from a submerged mycelial culture of Hericium erinaceus," Bioscience, Biotechnology and Biochemistry, vol. 67, no. 6, pp. 1292–1298, 2003.
[25] D. M. Kim, C. W. Pyun, H. G. Ko, et al., "Isolation of antimicrobial substances from Hericium erinaceum," Mycobiology, vol. 28, pp. 33–38, 2000.
[26] T. Mizuno, H. Saito, T. Nishitoba, et al., "Antitumor-active substances from mushrooms," Food Review International, vol. 11, pp. 23–61, 1995.
[27] S. Kuwahara, E. Morihiro, A. Nemoto, et al., "Synthesis and absolute configuration of a cytotoxic fatty acid isolated from the mushroom Hericium erinaceus," Bioscience, Biotechnology and Biochemistry, vol. 56, no. 6, pp. 1417–1419, 1992.
[28] C.-F. Liu, J.-N. Fang, X.-Y. Li, and X.-Q. Xiao, "Structural characterization and biological activities of SC4, an acidic polysaccharide from Salvia chinensis," Acta Pharmacologica Sinica, vol. 23, no. 2, pp. 162–166, 2002.
[29] J.-L. Mau, H.-C. Lin, and C.-C. Chen, "Antioxidant properties of several medicinal mushrooms," Journal of Agricultural and Food Chemistry, vol. 50, no. 21, pp. 6072–6077, 2002.
L. Lu, J. Li, and Y. Cang, “PCR-based sensitive detection of
F. Martin, A. Aerts, D. Ahren, A. Brun, H. Kawagishi, M. Ando, H. Sakamoto, S. Yoshida, F. Ojima, Y. Ishiguro, N. Ukai, and S. Furukawa, “Erinacines A, B and C, strong stimulators of nerve growth factor (NGF)-synthesis, from the mushroom Hericium erinaceum,” Tetrahedron, vol. 35, no. 10, pp. 1569–1572, 1994.

H. Kawagishi, M. Ando, K. Shinba, H. Sakamoto, S. Ojima, Y. Ishiguro, N. Ukai, and S. Furukawa, “Chromans, hericenones C, G and H from the mushroom Hericium erinaceum,” Phytochemistry, vol. 32, no. 1, pp. 175–178, 1992.

H. Kawagishi, M. Ando, H. Sakamoto, S. Yoshida, F. Ojima, Y. Ishiguro, N. Ukai, and S. Furukawa, “Hericenones D and E, stimulators of nerve growth factor (NGF)-synthesis, from the mushroom Hericium erinaceum,” Tetrahedron Letters, vol. 32, no. 35, pp. 4561–4564, 1991.

T. Mizuno, T. Wasa, H. Ito, C. Suzuki, and N. Ukai, “Antitumor-active polysaccharides isolated from the fruiting body of Hericium erinaceum, an edible and medicinal mushroom called yamabushitake or houtou,” Bioscience, Biotechnology, and Biochemistry, vol. 56, no. 2, pp. 347–348, 1992.

H. X. Wang and T. B. Ng, “A new laccase from dried fruiting bodies of the monkey head mushroom Hericium erinaceum,” Biochemical and Biophysical Research Communications, vol. 322, no. 1, pp. 17–21, 2004.

L. Lu, J. Li, and Y. Cang, “PCR-based sensitive detection of medicinal fungi hericium species from ribosomal internal transcribed spacer (ITS) sequences,” Biological and Pharmaceutical Bulletin, vol. 25, no. 8, pp. 975–980, 2002.

U. K. Laemmli and M. Favre, “Gel electrophoresis of proteins,” Journal of Molecular Biology, vol. 80, pp. 575–599, 1973.

F. Martin, A. Aerts, D. Ahrens, A. Brun, M. E. J. Danchin, F. Duchaussoy, I. Gibon, A. Kohler, E. Lindquist, V. Pereda, J. Labbe, Y. C. Lin, V. Legue, F. Le Tacon, R. Tejada, A. Deveau, S. DiFazio, C. Coutinho, C. Delaruelle, J. C. Detter, A. Deveau, S. Duplessis, L. Fraissinet-Tachet, E. Lucic, P. Frey-Klett, C. Coutinho, C. Delaruelle, J. C. Detter, A. Deveau, S. DiFazio, C. Zhuang, T. Sun, H. X. Wang, and T. B. Ng, “A novel lectin with potent antitumor, mitogenic and HIV-1 reverse transcriptase inhibitory activities from the edible mushroom Pleurotus citrinopileatus,” Biochimica et Biophysica Acta, vol. 1780, no. 1, pp. 51–57, 2008.

H. X. Wang and T. B. Ng, “Purification of a novel low-molecular-mass laccase with HIV-1 reverse transcriptase inhibitory activity from the mushroom Tricholoma giganteum,” Biochemical and Biophysical Research Communications, vol. 315, no. 2, pp. 450–454, 2004.

H. X. Wang and T. B. Ng, “An antifungal peptide from baby lima bean,” Applied Microbiology and Biotechnology, vol. 73, no. 3, pp. 576–581, 2006.

Q. Liu, H. Wang, and T. B. Ng, “Isolation and characterization of a novel lectin from the wild mushroom Xerocomus spadiceus,” Peptides, vol. 25, no. 1, pp. 7–10, 2004.

G. Q. Zhang, J. Sun, H. X. Wang, and T. B. Ng, “A novel lectin with antiproliferative activity from the medicinal mushroom Pholiota adiposa,” Acta biochimica Polonica, vol. 56, no. 3, pp. 415–421, 2009.

C. Zhuang, T. Murata, T. Usui, H. Kawagishi, and K. Kobayashi, “Purification and characterization of a lectin from the toxic mushroom Amanita pantherina,” Biochimica et Biophysica Acta, vol. 1291, no. 1, pp. 40–44, 1996.

X. Y. Ye, T. B. Ng, P. W. K. Tsang, and J. Wang, “Isolation of a homodimeric lectin with antifungal and antiviral activities from red kidney bean (Phaseolus vulgaris) seeds,” Journal of Protein Chemistry, vol. 20, no. 5, pp. 367–375, 2001.

O. Gozia, J. Ciopraga, T. Bentia, M. Lungu, I. Zamfirescu, R. Tudor, A. Roseanu, and F. Nitu, “Antifungal properties of lectin and new chinatines from potato tubers,” Comptes Rendus de l’Academie des Sciences. Series III, vol. 316, no. 8, pp. 788–792, 1993.

W. F. Broekaert, J. A. N. Van Parijs, F. Leyns, H. Joos, and W. J. Peumans, “A chitin-binding lectin from stinging nettle rhizomes with antifungal activities,” Comptes Rendus de l’Academie des Sciences. Series III, vol. 316, no. 8, pp. 788–792, 1993.

W. F. Broekaert, J. A. N. Van Parijs, F. Leyns, H. Joos, and W. J. Peumans, “A chitin-binding lectin from stinging nettle rhizomes with antifungal properties,” Science, vol. 245, no. 4922, pp. 1100–1102, 1989.

J. Ciopraga, O. Gozia, R. Tudor, L. Brezuica, and R. J. Doyle, “Fusarium sp. growth inhibition by wheat germ agglutinin,” Biochimica et Biophysica Acta, vol. 1428, no. 2-3, pp. 424–432, 1999.

M. Böttcher and F. Grosse, “HIV-1 protease inhibits its homologous reverse transcriptase by protein-protein interaction,” Nucleic Acids Research, vol. 25, no. 9, pp. 1709–1714, 1997.

H.-C. Hsu, C.-I. Hsu, R.-H. Lin, C.-L. Kao, and J.-Y. Lin, “Fipvvo, a new fungal immunomodulatory protein isolated from Volvariella volvacea,” Biochemical Journal, vol. 323, part 2, pp. 557–565, 1997.