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Original Article

Molecular cloning, expression, and characterization of a *Sophora alopecuroides* lectin from *Escherichia coli*

Yang Li[1], Tingting Li[1], Jinyao Li[1], Dongliang Liu[1], Jie Yang[1], Jianhua Yang[1,2], Fuchun Zhang[1], and Surong Sun[1*]

[1]Xinjiang Key Laboratory of Biological Resources and Genetic Engineering, College of Life Science and Technology, Xinjiang University, Urumuqi 830046, China
[2]Texas Children's Cancer Center, Department of Pediatrics, Dan L. Duncan Cancer Center, Baylor College of Medicine, Houston, TX 77030, USA
*Correspondence address. Tel: +86-991-8582077; Fax: +86-991-8583517; E-mail: sr_sun2005@163.com

Sophora alopecuroides **lectin (SAL) has been isolated from the seeds and confirmed to have antifungal and antitumor activities, and presently the preparation of the natural lectin was cumbersome, time-consuming, and the yield was relatively low for further analysis. In this study, the signal peptide of lectin, the modification sites, and the secondary structure were analyzed, and the three-dimensional structures of SAL were modeled. The gene of SAL was amplified by the reverse transcription polymerase chain reaction, and cloned into the pET-30a vector and expressed in *Escherichia coli* BL21(DE3) by the induction of isopropyl-beta-D-thiogalactopyranoside. Totally, 400 mg of recombinant SAL (rSAL) was purified from 1 l of bacterial culture through Ni-NTA agarose column and the purity reached 95%. The recombinant protein was further confirmed by western blot using rSAL-specific antibody. The biological activity analysis results showed that rSAL exclusively bound to D-galactose and had universal hemagglutinating activities to human A, B, O, and AB, and rabbit and mouse erythrocytes. rSAL also inhibited the growth of fungi, the proliferation of cancer cells, and the HIV-I reverse transcriptase activity. In conclusion, this study indicates that rSAL can be produced in large quantities in the prokaryotic expression system and the recombinant protein still retains the various biological activities, which will make the large-scale production of SAL recombinant protein at dramatically reduced cost possible.**

Keywords SAL cloning; prokaryotic expression; purification; biological activities

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Introduction

Lectins are a class of non-covalent, carbohydrate-binding proteins of non-immune origins possessing at least one non-catalytic domain. They can reversibly recognize and bind to monosaccharides or oligosaccharides [1]. Legume lectins have a variety of biological functions. Many studies have shown that legume lectins can interfere with the generation of fungi cell walls and affect the normal metabolism of fungal cells [2]. Other functions of legume lectins include identifying carbohydrates on glycoproteins, glycopeptides, and biomembranes, inducing expression of some defense genes in plants [3], resisting invasion of pathogens and germination of conidia [4], and functioning as plant storage proteins or microbial symbiosis media, etc. Transgenic crops which express the legume lectin genes showed enhanced insect resistance and antibacterial ability [5]. Legume lectins isolated from Chinese black soybeans, haricot beans (*Phaseolus vulgaris*), and capers (*Capparis spinosa*) can inhibit the proliferation of tumor cells [6–8] and lectin from French beans has been demonstrated to have anti-HIV activity [9]. In recent years, a large amount of research has been conducted on the structure and function of legume lectins. The application of legume lectins in biology, agronomy, and biomedical science has drawn extensive attention.

Sophora alopecuroides belongs to the *Sophora* genus, Leguminosae plant family. The *S. alopecuroides* is a medicinal plant widely distributed in the desert areas of Northwest China. Previous studies have shown that the root, stem, seeds, and whole plant of *S. alopecuroides* could be used as medicine [10]. *Sophora alopecuroides* has the following characteristics: bitter in taste, detoxification activity when heated, and insecticidal and analgesic activities [11]. Our previous study demonstrated that the lectin isolated from the seeds of *S. alopecuroides* has antifungal and antitumor activities [11]. However, the preparation procedure of the natural lectin was cumbersome, time-consuming, and with relatively low yields. Large amount of *S. alopecuroides* lectin (SAL) was needed for the further study of the properties and clinical applications of SAL. Recently, efforts have been focused on the development of new technologies to produce SAL, such as recombinant technology.

In the current study, we analyzed the signal peptide of lectin, its modification sites, and the secondary structure, and modeled the three-dimensional structures of SAL. In addition, SAL gene was amplified by the reverse transcription polymerase chain reaction (RT-PCR), and cloned into an efficient expression vector to produce SAL in *Escherichia coli*. Recombinant SAL (rSAL) with His-tag was purified through Ni-NTA agarose column and its hemagglutination, antifungal, antitumor, and anti-HIV-I reverse transcriptase activities were investigated.

Material and Methods

Materials

Sophora alopecuroides was obtained from Hong Yanchi Reservoir (Urumuqi, China). Trizol, DNA marker, Taq DNA polymerase, T4 DNA ligase, limited incision enzyme, and DNA preparation kits were from TaKaRa (Dalian, China). D-seminose, D-sorbol, *N*-Ac-D-aminoglucose, D-galactose, and thyroid protein were from Sigma Company (St. Louis, MO, USA). pET-30a vector, pCR2.1 vector, and *E. coli* strains (DH5α and BL21) were from Invitrogen (Beijing, China). *Penicillium italicum*, *Alternaria alternate*, *Rhizopus stolonifera*, *Botrytis cinerea* Pers, the Vero (African green monkey kidney) cell line, esophagus carcinoma (Eca-109) cell line, and cervical carcinoma (HeLa) cell line were from Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China).

Structural analysis of SAL

Analysis of the basic characteristics of SAL was done by the ExPASy Proteomics Server software (http://www.expasy.org/). The signal peptide of SAL was predicted by SignalP server, while its modification site was acquired from PROSCAN software (http://npsa-pbil.ibcp.fr/). Secondary structural analysis was accomplished using the SOPMA software (http://npsa-pbil.ibcp.fr). In addition, three-dimensional structure of SAL was constructed using SWISS-MODEL server (http://swissmodel.expasy.org/).

SAL gene cloning and identification

We first analyzed the codon usage of SAL gene sequence for expression in *E. coli* by using Codon Usage Database (http://www.kazusa.or.jp/codon/). Fresh leaves of *S. alopecuroides* were powdered with pestle in liquid nitrogen. Total cellular RNA was isolated by using RNA Extraction Kit (TaKaRa) and the cDNA was reversely transcribed by TransScript First-Strand cDNA Synthesis SuperMix (TransGen Biotech, Beijing, China). The open reading frame of SAL gene was amplified by PCR with the gene-specific forward primer (5′-ATGGCCA TATTCCAGAAACAC-3′) and reverse primer (5′-TCACAC AACACTTGCCATATA-3′) designed according to the published sequence (GenBank accession No. DQ011517.1). DNA amplification was performed under the following conditions: 94°C for 5 min, 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min (35 cycles). The PCR products were purified and digested sequentially with *Nco*I and *Xho*I, and then inserted into pCR2.1 vector. Restriction enzyme digestion and sequence analysis were used to identify the positive clones.

DNA fragment encoding mature SAL protein was amplified and isolated by PCR from the plasmid pCR2.1-SAL with gene-specific primers (5′-CATGCCATGGCAGACT CACTCTCCTTCACCT-3′ and 5′-CCGCTCGAGCACAAC ACTTGCCATATACAT-3′). The conditions of PCR were described above. The PCR product was purified and digested sequentially with *Nco*I and *Xho*I, and then inserted into pET-30a(+) vector. After the sequence was verified, the constructed plasmid was transformed into the *E. coli* BL21(DE3).

Expression, purification, and analysis of the recombinant protein

The optimized expression of the recombinant protein was induced with 0.3 mM isopropyl-beta-D-thiogalactopyranoside (IPTG) at 28°C for 4 h. The bacterial pellet obtained from 1 l of liquid culture (8000 *g* for 10 min at 4°C) was suspended in 50 ml buffer (10 mM PBS and 0.5 M NaCl, pH 8.0). The cells were lysed by sonication on ice using an ultrasonic cell disruptor (Cole-Parmer Instrument, Chicago, USA). The lysate was centrifuged at 12,000 *g* for 25 min at 4°C. The supernatant containing soluble cellular material (total volume of 45 ml) was used for purification by immobilized metal affinity chromatography (IMAC). The column containing 5 ml of Ni^{2+}-NTA affinity resin was equilibrated with 10 column volumes (CV) of buffer (10 mM PBS, 0.5 M NaCl, and 10 mM imidazole, pH 8.0). The supernatant was loaded into the column by gravity and the unbounded proteins were washed with 10 CV of buffer (20 mM PBS, 0.5 M NaCl, and 20 mM imidazole, pH 7.4) [12]. The bounded fusion protein was eluted with 5 CV of buffer (20 mM PBS, 0.5 M NaCl, and 250 mM imidazole, pH 8.0). The protein samples were run on a 15% tricine–SDS–PAGE and transferred onto a nitrocellulose membrane (Pall, New York, USA). The membrane was blocked with 3% (v/v) BSA/TBST buffer overnight at 4°C. The membrane was washed for three times (5 min each) with TBST buffer and incubated with anti-His tag primary antibody (1 : 1000) in 1% (v/v) BSA/TBS buffer for 2 h at 37°C. The membranes were then washed three times with TBST buffer and incubated with horseradish peroxidase-conjugated secondary antibodies (Santa Cruz, USA) at 37°C for 1 h. After washing, the membrane was analyzed using the ECL chemiluminescent kit (Invitrogen) [13].

Assays of hemagglutinating activity

In the hemagglutinating activity assay, a serial 2-fold dilution of the lectin solution in microtiter U-plates (25 μl) was mixed

with 25 μl of a 2% suspension of rabbit red blood cells in PBS (pH 7.2) at 20°C. The results were recorded after ~1 h when erythrocytes in the blank had fully sedimented. The hemagglutination titer, defined as the reciprocal of the highest dilution exhibiting hemagglutination, was reckoned as 1 hemagglutination unit. Hemagglutination assays were carried out using normal rabbit and human ABO group erythrocytes according to Chan *et al*. [14].

Inhibition of hemagglutinating activity by different carbohydrates

Inhibition of hemagglutinating activity by various carbohydrates was performed as described by Wong *et al*. [15]. rSAL solution (25 μl) was added to a well in a 96-well microtiter plate and serially diluted with 25 μl of PBS. Saccharide solution (50 μl) was then added and mixed with the rSAL solutions in the wells. After 30 min of incubation at room temperature, 50 μl of erythrocytes was added to each well. The plate was then allowed to stand at room temperature for about an hour before the results were observed. The carbohydrates for the inhibition test included trehalose, D-xylose, maltose, D-mannose, sucrose, L-arabinose, glucose, D-galactose, methy-α-D-galactose, and ovalbumin.

Effects of temperature and pH on lectin hemagglutinating activity

To test the effect of temperature on the hemagglutinating activity of rSAL, lectin samples were incubated at various temperatures (30–100°C at 10°C intervals) for 20 min, cooled to room temperature, and then the residual hemagglutinating activities were measured as described above.

To test the effect of pH on the hemagglutinating activity of rSAL, the lectin samples were incubated in buffers of various pH from 4 to 11 (TBS buffer was used as the control) and kept for 1 h at room temperature. And then, the lectin samples were adjusted back to TBS and the residual agglutinating activities were assayed as described above.

Effect of metal ions on lectin hemagglutinating activity

The effect of different metal ions on the lectin hemagglutinating activity was tested by the method described by Suzuki *et al*. [16]. A sample (100 μg/ml) was incubated in 10 mM ethylenediaminetetraacetic acid (EDTA) for 1 h at room temperature, dialyzed against PBS, and titrated. To the demetalized rSAL, 0.1 M metal cation ($CaCl_2$, $MgCl_2$, or $ZnCl_2$) was added, and the solution was incubated for 1 h at room temperature and then titrated.

Antifungal activity of rSAL

The antifungal activities of purified lectin towards *P. italicum*, *A. alternate*, *R. stolonifera*, and *B. cinerea* Pers were carried out in 90 × 12 mm^2 Petri dishes containing 20 ml of potato dextrose agar (PDA). Suspensions (100 ml) of fungal spores were added to the PDA, sterile paper disks (6 mm in diameter) were placed on the agar, and 20 ml of the purified lectin solution was added onto each disk. The dishes were incubated at 28°C for 72 h until mycelia growth enveloped the control disks. The sizes of inhibition zones were observed and their diameters were measured. In order to obtain a quantitative evaluation of the antifungal activity of SAL, following the method of Park *et al*. [17], equal volume of different protein concentrations was added into a 96-well microtiter plate containing 100 μl of suspension of fungal spores (2.5×10^4 spores/ml), and incubated at 28°C for 24 h. Fungal growth was evaluated by measuring the optical density of the culture at 595 nm using a Benchmark Plus 10365 microplate reader (Bio-Rad, Hercules, USA). Growth inhibition was calculated by following formula:

$$\text{Inhibitory ratio}\,(\%) = [1 - \text{OD}_{595(\text{lectin})}]/\text{OD}_{595(\text{control})} \times 100\%$$

IC_{50} was defined as the protein concentration at which 50% inhibition was reached [11].

Cytotoxicity assay

Tests were prepared according to Zhang *et al*. [18] and Mosmann [19]. Tumor cells in logarithmic growth phase were seeded at 5×10^4 cells per well independently in a 96-well plate at a final volume of 100 μl. The wells with Dulbecco's modified Eagle's medium (DMEM) complete medium were set as the blank control group. These plates were incubated at 37°C for 24 h. After removal of the culture medium, 100 μl of rSAL (0.334, 0.668, 1.336, 2.004, and 2.772 mg) dissolved in DMEM was added into each well for the indicated times. MTT colorimeter was utilized to detect the anticancer activity of lectin.

Inhibitory activity of rSAL against HIV-I reverse transcriptase

The inhibitory effect of rSAL against HIV-I reverse transcriptase (HIV-I RT) was carried out by using an enzyme-linked immunosorbent assay kit from Roche (Mannheim, Germany) as described by Zhang *et al*. [20]. By detecting the absorbance values, the inhibitory ratio of rSAL on HIV-I RT activity was calculated as follows:

$$\text{Inhibitory ratio}\,(\%) = \{[\text{OD}_{490(\text{blank})} - \text{OD}_{490(\text{control})}] - [\text{OD}_{490(\text{lectin})} - \text{OD}_{490(\text{blank})}]\}/[\text{OD}_{490(\text{control})} - \text{OD}_{490(\text{blank})}] \times 100\%$$

Statistical analysis

All the results presented here were confirmed in at least three independent experiments. These data were expressed as

mean ± SD. Statistical comparisons were made by Student's *t*-test. $P < 0.05$ was considered of significant difference.

Results

Structural analysis of SAL

The molecular weight and isoelectric point (pI) of SAL were 31,181 Da and 6.25, respectively, in terms of ExPASy Proteomics Server software. The amino acid sequence analysis of SAL showed that the components of the acidic amino acids and the hydrophobic amino acids were 9.28% and 43.2%, respectively. The grand average of hydrophobicity of SAL was −0.151. Therefore, a preliminary conclusion could be drawn that SAL was a hydrophilic protein.

The signal peptide prediction results showed that the SAL primarily existed as an inactive precursor, the original protein (proprotein) form, become the mature form (signal peptide-removed protein) after processing, and then it is secreted to the outside of the cell to function, so the signal peptide (Met1-Ser29) guided SAL to cross membrane and locate in the cells of a particular area.

The amino acid sequence of deduced SAL protein showed that the sequence may contain an N-glycosylation site (Asn209-Val212), three protein kinase C-phosphorylation sites (Thr93-Arg95, Thr180-Lys182, and Thr198-Arg200), eight casein kinase II phosphorylation sites (Thr36-Asp39, Thr64-Asp67, Ser97-Glu100, Ser113-Asp116, Thr128-Glu131, Thr145-Asp148, Thr165-Asp168, and Ser245-Glu248), a tyrosine kinase phosphorylation site (Lys268-Tyr275), and five N-nutmeg acylation sites (Gly26-Asp31, Gly132-Tyr137, Gly135-Leu140, Gly175-Thr180, and Gly194-Ala199). The details were shown in **Supplementary Fig. S1**. These post-translation modifications can be accomplished for the natural protein, however, it may not happen in the rSAL expressed by prokaryotic system, thus the biological activities of rSAL may be affected as compared with the natural protein.

The secondary structure prediction results showed that the α-helix, β-sheet, β-turn, and random coil content of SAL were ~16.07%, 32.14%, 6.79%, and 45%, respectively, which are similar to those of *Philyra pisum* lectin (PPL). It was estimated that the α-helix, β-sheet, β-turn, and random coil contents of PPL were 14.0%, 39.6%, 15.8%, and 30.6%, respectively [21]. It is worth noting that both of the two kinds of lectins have low α-helix and high β-sheet contents. Previous investigation indicated that PPL exerted hemagglutinating activity and antiproliferative activity against human lung cancer cells [21]. Thus, we speculated that SAL might have hemagglutinating activity and antitumor activity as well.

The comparison of amino acid sequence of SAL with other leguminous lectins showed that SAL shared the highest sequence similarity of 99% with *Sophora flavescens* lectin (SFL) (**Supplementary Fig. S2**), and the three-dimensional structure of SAL was successfully simulated by homology modeling tool regarding SFL as template. The predicted three-dimensional structure model of SAL is presented in **Fig. 1**, which is very similar to that of SFL (**Supplementary Fig. S2**). It was shown that SAL consisted of six strands of the back β-sheet (Back β1−β6) and seven strands of the front β-sheet (Front β1−β7). These sheets were connected by five smaller sheets (S-sheet) [22]. This structure is similar to a flat bell-shaped. A small pocket in the front is sugar-binding sites, which contribute to binding to divalent metal ion, and the main hydrophobic core is between Back β-sheet and Front β-sheet. This is consistent with the previous finding in SFL [22]. It has been reported that SFL could inhibit apparently the growth of the mycelium of *Gibberlla saubinetii* Sacc., *Pyricularia oryzae* Cav., and *Fusarium vasinfectum* Atk, inhibit the growth of MCF-7 cells and HeLa cells, and induce apoptotic morphology in MCF-7 cells without affecting human normal mammary epithelial MCF-10A cells [23]. Therefore, we reasonably speculated that the SAL may also have the antifungal and antitumor activities.

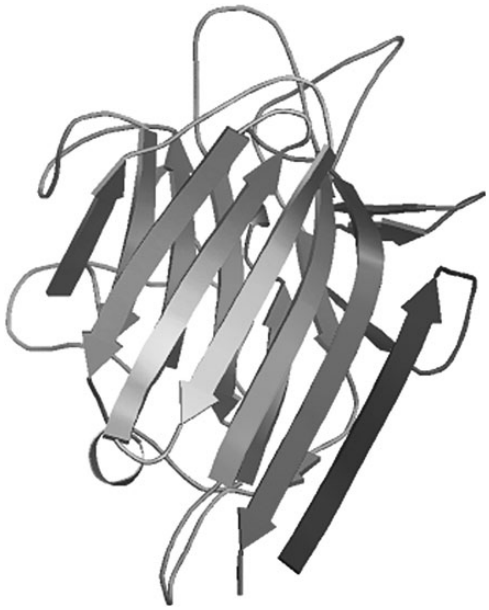

Figure 1. Ribbon diagram of the SAL The overall structure of the protein is shown as a ribbon model (Showed by chainbows). The six strands of the back β-sheet (Back β1−β6) and seven strands of front β-sheet (Front β1−β7), these sheets were connected by five smaller sheets (S-sheet). This structure is similar to a flat bell-shaped.

Expression and purification of rSAL

The *E. coli* strain transfected by pET30a-SAL was grown at 28°C and induced by 0.3 mM IPTG for 4 h. Then, the total soluble proteins were extracted and SDS−PAGE was used to identify the induced protein. The results showed that a protein band of ~40 kDa was induced after adding IPTG, indicating that His-tagged rSAL (~40 kDa, which was consistent with the calculated one) was produced (**Fig. 2**). Totally, 400 mg of

rSAL was purified from 1 l bacterial culture by a Ni-NTA agarose column and the purity of rSAL reached 95% as calculated by the grayscale reading (**Fig. 2**). In our knowledge, this is the highest yield reported so far for a recombinant lectin [10]. Western blot was used to identify the recombinant protein with a mouse anti-His monoclonal antibody and a single band of ~40 kDa was detected, indicating that the His-tagged rSAL has been correctly expressed in *E. coli*.

Hemagglutinating activity and sugar-binding activity of rSAL

In order to check whether the rSAL had the biological functions, the activities of hemagglutination and sugar binding were examined. As shown in **Table 1**, rSAL exhibited hemagglutinating activities on rabbit and mouse blood cells as well as human ABO type blood sample. rSAL showed the strongest hemagglutinating activity on rabbit blood cells, in which its minimum effective concentration was 0.0056 mg/ml.

For sugar inhibition experiments, 14 kinds of sugars were tested and only 0.05 M D-galactose can strongly inhibit the hemagglutinating activity of 0.022 mg/ml rSAL, indicating that D-galactose was the specific binding sugar of lectin.

pH and temperature stability of rSAL

The hemagglutinating activity of rSAL was tested under different pH and temperature conditions. As shown in **Fig. 3A**, the hemagglutinating activity of rSAL is stable within the pH range of 6–10, and the hemagglutinating activity still kept 60% when the pH value was lower than 5 or higher than 11. The hemagglutinating activity remained stable in the temperature range of 30–80°C, and 65% of the hemagglutinating activity was even observed at 100°C (**Fig. 3B**). Our results indicated that rSAL was stable under broader range of pH and temperature.

The hemagglutinating activity of rSAL can be inhibited by the treatment of EDTA, which can be restored by 0.04 M Mn^{2+}, while Ni^{2+}, Cu^{2+}, and Hg^{2+} can reduce its hemagglutinating activity (data not shown), indicating that rSAL is a Mn^{2+}-dependent protein.

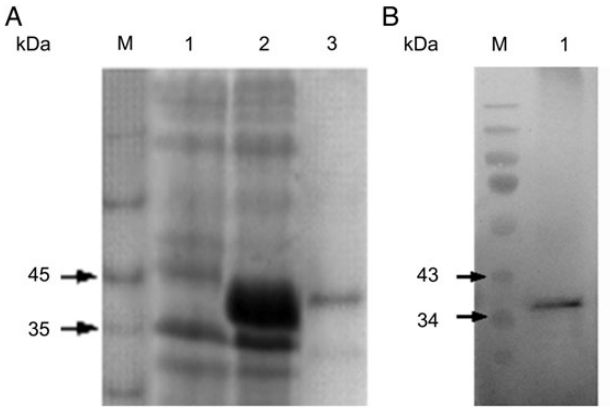

Figure 2. Expression and purification of the rSAL fusion protein (A) Tricine–SDS–PAGE (15%). Lane M, protein molecular mass marker (14.4–116 kDa); lane 1, initial crude extract of *E. coli* proteins without the fusion protein His-rSAL; lane 2, the total proteins of pET30a/BL21 cells were extracted after being induced by 0.3 mM IPTG; lane 3, IMAC purification, elution with 250 mM imidazole, the purity was 95%. (B) Western blot analysis of rSAL using anti-his tag monoclonal antibody. Lane 1, western blotting analysis of purified fusion protein His-rSAL.

Table 1. Hemagglutinating activity analysis of rSAL against human and animal erythrocytes

Group		Concentration (mg/ml)[a]
Human blood cells	A	0.02
	B	0.02
	AB	0.02
	O	0.02
Animal blood cells	New Zealand rabbit	0.0056
	KM mouse	0.14

[a]rSAL concentration for minimum hemagglutinating.

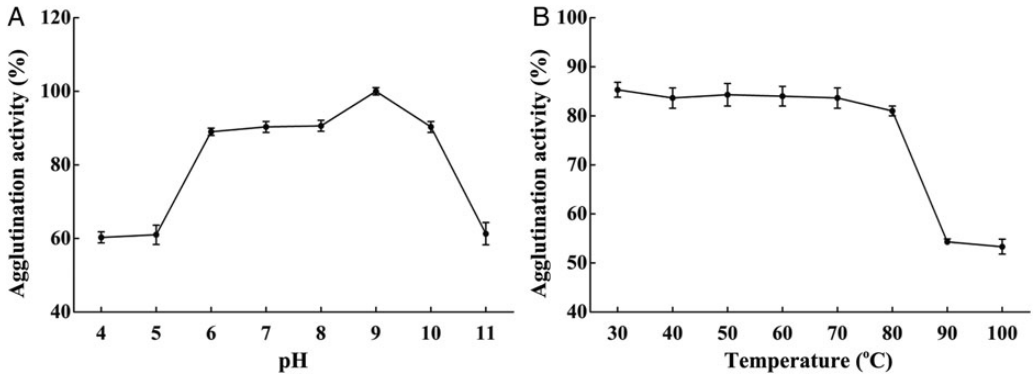

Figure 3. Effects of pH and temperature on the hemagglutinating activity of rSAL Towards rabbit erythrocytes suspension in TBS, full activity (100%) corresponds to 0.011 mg/ml of rSAL. (A) Effect of pH on the hemagglutinating activity of rSAL towards rabbit erythrocyte suspension in TBS. (B) Effect of temperature on the hemagglutinating activity of rSAL towards rabbit erythrocyte suspension in TBS.

Antifungal activity of rSAL

rSAL had a growth inhibitory effect on four species of fungi as shown in **Fig. 4**. The IC_{50} of rSAL on *R. stolonifera*, *B. cinerea* Pers, and *A. alternate* was 0.297, 0.367, and 0.635 mg/ml, respectively. It was also observed that the growth of *P. italicum* was inhibited by 41.35% when 0.8 mg/ml rSAL was applied. These results suggest that rSAL has the strongest inhibitory effect on *R. stolonifera*, followed by *B. cinerea* Pers, *A. alternate*, and *P. italicum*. rSAL's growth inhibitory effect on *R. stolonifera* was significantly higher than those on other three fungi ($P < 0.05$).

Cytotoxic effect of rSAL on tumor cells

The inhibitory effect of rSAL on cell proliferation was measured by MTT assay. rSAL exerted a potent cytotoxic effect on Eca-109 and HeLa cells ($P < 0.05$) with IC_{50} of 14.22 and 12.20 mg/ml, respectively. As shown in **Fig. 5**, the inhibition is dose dependent for these two cell lines. Its effect on normal monkey kidney Vero cells was significantly lower than those on other two cancer cell lines ($P < 0.01$).

Inhibitory activity of rSAL to HIV-I RT

HIV-I RT activity was measured in the presence of varying concentrations of rSAL and a maximum inhibition of 58.17% was observed with an estimated IC_{50} of 0.24 mg/ml for rSAL (**Table 2**).

Discussion

SAL, a galactose-binding plant lectin, has attracted much attention due to its remarkable antitumor and antiviral activities. However, the lectin extraction efficiency was low based on conventional biological methods to purify natural lectin, and the extraction process was complicated. New methods need to be developed for large-scale preparation of lectin within a short time at low cost, such as gene recombinant techniques [24]. In this study, an *E. coli* prokaryotic expression system was employed to express the SAL gene and rSAL with His_6 tag was efficiently produced. rSAL was purified by metal Ni affinity chromatography. We found that both the purified rSAL and the natural SAL (nSAL) had similar biological activities. A large amount of SAL can be produced by the genetic engineering method that does not affect its biological activity.

nSAL has been found to be stable in its hemagglutinating activity within a temperature of 30–90°C and a pH of 7–11 [11]. The results of this study showed that for the rSAL, the optimum pH was 6–10, and the optimum temperature for its hemagglutinating activity was 30–80°C. In comparison with that of nSAL, the hemagglutinating activity of rSAL was weaker. rSAL was more stable than lectins from French bean (0–50°C, pH 6–8) [9], *Astragalus mongholicus* (0–60°C, pH 4.5–7.5) [25], and *Curcuma longa* (0–40°C, pH 6–7) [26].

A previous study on lectin from *Canavalia ensiformis* seed showed that the purified lectin could agglutinate only

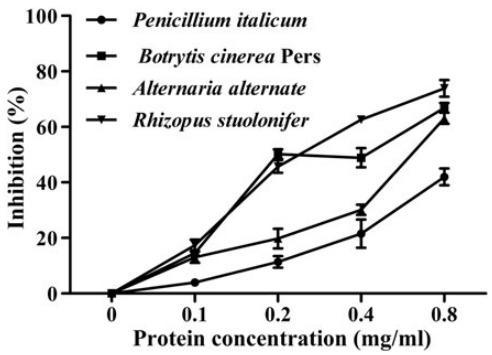

Figure 4. Inhibitory activity of rSAL against four kinds of fungi Controls for 0% inhibitory ratio (blank) were determined in PBS buffer. Values shown are the means of three independent experiments performed on different occasions with error bars representing standard deviations. Percentage of growth inhibition was measured at different concentrations of SAL with the following test organisms: *P. italicum*, *A. alternate*, *R. stolonifera*, and *B. cinerea* Pers.

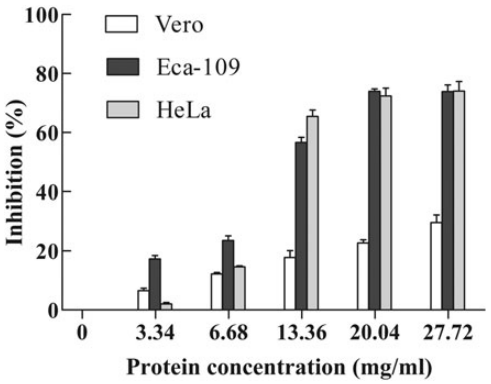

Figure 5. Inhibitory effects of rSAL on the proliferation of HeLa, Eca-109, and Vero cells The cell death rate was measured by MTT assay using HeLa and Eca-109 cells. Results are presented as % inhibition, as compared with the DMEM complete medium control group. Data are expressed as mean ± SD from three experiments

Table 2. HIV-I RT inhibitory activity of rSAL

rSAL (μg/ml)	RT activity[a]	Inhibition (%)[b]
Control	0.265 ± 0.010	–
1.5	0.200 ± 0.024	25.23
3	0.156 ± 0.004	41.96
6	0.142 ± 0.003	47.63
12	0.130 ± 0.028	52.25
24	0.114 ± 0.007	58.17

[a]Date represent mean values of OD for three independent experiments (mean values ± SD).

[b]The percentage of viable treated cells was calculated relative to untreated controls.

rabbit erythrocytes [27]. In our study, rSAL showed hemagglutinating activities on rabbit and mouse blood cells, and human ABO type blood cells. Its hemagglutinating activity on rabbit blood cells was significantly higher than that on other types of blood cells, which is similar to nSAL [11]. Others have found that lectin not only can agglutinate red blood cells, but also can agglutinate other cells, such as sperms, lymphocytes, and bacteria, etc. [28]. *Phaseolus coccineus* lectin had agglutination activities on tumor cells and certain microbes (such as *E. coli* and yeast) depending on the cell types [29].

In the sugar-binding assay, we found that rSAL can specifically bind D-galactose, and it was a Mn^{2+}-dependent protein, which is similar to nSAL [11]. The results indicated that the rSAL expressed in prokaryotic system did not change its sugar chain recognition specificity and metal ion binding capacity. This property suggests that rSAL can be used as a molecular probe or sugar-specific probe to study sugar chain structure on cell membranes and cell variation, and to study specific membrane receptors.

Published data have shown that only a few lectins have significant antifungal activity, such as lectins isolated from French bean [9] and flageolet beans [30]. Other lectins have no antifungal activity, such as lectins isolated from *P. vulgaris* cv [31] and *Bauhinia variegata* [32]. This study revealed that the inhibitory effect of rSAL on *A. alternate* (IC_{50} was 0.635 mg/ml) was similar to nSAL (IC_{50} was 0.125 mg/ml) [11]. rSAL had similar antifungal properties with nSAL. The recombinant protein had little difference from the native protein in its antibacterial properties [29]. Similar to other galactose-binding lectins, rSAL may inhibit pathogen infection and growth through binding to heterogeneous polysaccharides on the fungal surface. Further study on the antifungal mechanism of lectin from *S. alopecuroides* will provide basis for its use as a biological pesticide candidate to protect the crops from pests.

It has been reported that some lectins can inhibit the growth of tumor cells, such as lectins from Extralong Autumn Purple bean and French bean [31]. However, many lectins cannot inhibit the growth of tumor cells such as lectins isolated from *A. mongholicus* [25], *C. longa* L [26], and *Oudemansiella radicata* [33]. Our results indicated that rSAL can inhibit the growth of cancer cells, but its inhibitory effects on Eca-109 and HeLa cells (IC_{50} were 14.22 and 12.20 mg/ml, respectively) were lower than those of nSAL (IC_{50} were 0.7 and 0.5 mg/ml, respectively). So far, only a few studies have shown that plant lectins have inhibitory activity on animal viruses. Our study revealed that, similar to French bean hemagglutinin [9], rSAL could effectively inhibit HIV-I RT activity, indicating that lectin has a potent anti-HIV activity.

In summary, we made structure-based activity prediction for SAL and obtained rSAL by a prokaryotic expression system, and the recombinant protein has biological activities under broader range of pH and temperature. The results showed that the prokaryotic system can be used to produce SAL with biological activities. This method provides us with a new and effective method to obtain a large amount of rSAL and overcome the problems faced in the extraction of nSAL, and provides a basis for the in-depth study of SAL and its applications in the future.

Supplementary Data

Supplementary Data are available at *ABBS* online.

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References

1. Sharon N and Lis H. Legume lectins—a large family of homologous proteins. FASEB J 1990, 14: 198–208.
2. Murdock LL and Shade RE. Lectins and protease inhibitors as plant defenses against insects. J Agric Food Chem 2002, 50: 6605–6611.
3. Larroque M, Ramirez D, Lafitte C, Borderies G, Dumas B and Gaulin E. Expression and purification of a biologically active *Phytophthora parasitica* cellulose binding elicitor lectin in *Pichia pastoris*. Protein Expr Purif 2011, 80: 217–223.
4. Araújo-Filho JH, Vasconcelos IM and Martins-Miranda AS. A ConA-like lectin from *Dioclea guianensis Benth* has antifungal activity against *Colletotrichum gloeosporioides*, unlike its homologues, ConM and ConA. J Agric Food Chem 2010, 58: 4090–4096.
5. Melander M, Ahman I, Kamnert I and Strömdahl AC. Pea lectin expressed transgenically in oilseed rape reduces growth rate of pollen beetle larvae. Transgenic Res 2003, 12: 555–567.
6. Lin P, Ye X and Ng TB. Purification of melibiose-binding lectin from two cultivars of Chinese black soybeans. Acta Biochim Biophys Sin (Shanghai) 2008, 40: 1029–1038.
7. Wong JH and Ng TB. A homotetrameric agglutinin with antiproliferative and mitogenic activities from haricot beans. J Chromatogr B Analyt Technol Biomed Life Sci 2005, 828: 130–135.
8. Lam SK, Han QF and Ng TB. Isolation and characterization of a lectin with potentially exploitable activities from caper (*Capparis spinosa*) seeds. Biosci Rep 2009, 29: 293–299.
9. Lam SK and Ng TB. Isolation and characterization of a French bean hemagglutinin with antitumor, antifungal, and anti-HIV-1 reverse

transcriptase activities and an exceptionally high yield. Phytomedicine 2010, 17: 457–462.
10. Tang HS, Zhu K, An R and Sun SR. Preliminary study of antibacterial activity of protein crude extracts of *Sophora alopecuroides*. Xinjiang Agric Sci 2009, 46: 425–429.
11. Li TT, Yin XL, Liu DL, Ma XJ, Lv H and Sun SR. Isolation and characterization of a novel lectin with antifungal and antiproliferative activities from *Sophora alopecuroides* seeds. Acta Biochim Biophys Sin (Shanghai) 2012, 44: 606–613.
12. Larroque M, Ramirez D, Lafitte C, Borderies G, Dumas B and Gaulin E. Expression and purification of a biologically active *Phytophthora parasitica* cellulose binding elicitor lectin in *Pichia pastoris*. Protein Expr Purif 2011, 80: 217–230.
13. Xia L, Liu Z, Ma J, Sun S, Yang J and Zhang F. Expression, purification and characterization of cecropin antibacterial peptide from *Bombyx mori* in *Saccharomyces cerevisiae*. Protein Expr Purif 2013, 90: 47–54.
14. Chan YS, Wong JH, Fang EF, Pan W and Ng TB. Isolation of a glucosamine binding leguminous lectin with mitogenic activity towards splenocytes and anti-proliferative activity towards tumor cells. PLoS ONE 2012, 7: e38961.
15. Wong JH, Wan CT and Ng TB. Characterisation of a haemagglutinin from Hokkaido red bean (*Phaseolus vulgaris* cv. Hokkaido red bean). J Sci Food Agric 2010, 90: 70–77.
16. Suzuki T, Amano Y, Fujita M, Kobayashi Y, Dohra H, Hirai H and Morita T, *et al*. Purification, characterization, and cDNA cloning of a lectin from the mushroom *Pleurocybella porrigens*. Biosci Biotechnol Biochem 2009, 73: 702–709.
17. Park SC, Lee JR, Shin SO, Jung JH, Lee YM, Son H and Park Y, *et al*. Purification and characterization of an antifungal protein, C-FKBP, from Chinese cabbage. J Agric Food Chem 2007, 55: 5277–5281.
18. Zhang GQ, Sun J, Wang HX and Ng TB. A novel lectin with antiproliferative activity from the medicinal mushroom *Pholiota adiposa*. Acta Biochim Pol 2009, 56: 415–421.
19. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983, 65: 55–63.
20. Zhang GQ, Tian T, Liu YP, Wang HX and Chen QJ. A laccase with antiproliferative activity against tumor cells from a white root fungus *Abortiporus biennis*. Process Biochem 2011, 46: 2336–2340.
21. Byung TP, Byung SK and Heajin P. Binding Specificity of *Philyra pisum* lectin to pathogen-associated molecular patterns, and its secondary structure. Korean J Physiol Pharmacol 2013, 17: 547–551.
22. Prabu MM, Suguna K and Vijayan M. Variability in quaternary association of proteins with the same tertiary fold: a case study and rationalization involving legume lectins. Proteins Struct Funct Genet 1999, 35: 58–69.
23. Shi Z, Chen J, Li CY, An N, Wang ZJ, Yang SL and Huang KF. Antitumor effects of concanavalin A and *Sophora flavescens* lectin *in vitro* and *in vivo*. Acta Pharmacol Sin 2014, 35: 248–256.
24. Li CY, Luo P, Liu JJ, Wang EQ, Li WW, Ding ZH and Mou L. Recombinant expression of *Polygonatum cyrtonema* lectin with anti-viral, apoptosis-inducing activities and preliminary crystallization. Process Biochem 2011, 46: 533–542.
25. Yan QJ, Jiang ZQ, Yang SQ, Deng W and Han LJ. A novel homodimeric lectin from *Astragalus mongholicus* with antifungal activity. Arch Biochem Biophys 2005, 442: 72–81.
26. Petnual P, Sangvanich P and Karnchanatat A. A lectin from the rhizomes of turmeric (*Curcuma longa* L.) and its antifungal, antibacterial, and α-glucosidase inhibitory activities. Food Sci Biotechnol 2010, 19: 907–916.
27. Kwang SR and Na YP. Characterization of the lectin purified from *Canavalia ensiformis* shoots. Biotechnol Bioprocess Eng 2005, 10: 334–340.
28. Koehler JK. Lectins as probes of the spermatozoon surface. Arch Androl 1981, 6: 197–217.
29. Shi W. The biological activities of runner bean lectin. J Biol Chem 1995, 11: 567–573.
30. Xia L and Ng TB. An antifungal protein from flageolet beans. Peptides 2005, 26: 2397–2403.
31. Fang EF, Lin P, Wong JH, Tsao SW and Ng TB. A lectin with anti-HIV-1 reverse transcriptase, antitumor, and nitric oxide inducing activities from seeds of *Phaseolus vulgaris* cv. extralong autumn purple bean. J Agric Food Chem 2010, 58: 2221–2229.
32. Lin P and Ng TB. Preparation and biological properties of a melibiose binding lectin from *Bauhinia variegata* seeds. J Agric Food Chem 2008, 56: 10481–10486.