Purification of melibiose-binding lectins from two cultivars of Chinese black soybeans

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A dimeric 50 kDa melibiose-binding lectin was isolated from the seeds of the cultivar of soybean (*Glycine max*), called the small glossy black soybean. The isolation procedure comprised ion exchange chromatography on Q Sepharose, SP Sepharose and Mono Q followed by gel filtration on Superdex 75. The lectin was adsorbed on all three ion exchangers, and it exhibited an N-terminal sequence identical to that of soybean lectin. Of all the sugars tested, melibiose most potently inhibited the hemagglutinating activity of the lectin, which was stable between pH 3−12 and 0−70 °C. The lectin evoked maximal mitogenic response at about the same molar concentration as Con A. However, the response was much weaker. The soybean lectin inhibited the activity of HIV-1 reverse transcriptase as well as the proliferation of breast cancer MCF7 cells and hepatoma HepG2 cells with an IC₅₀ of 2.82 µM, 2.6 µM and 4.1 µM, respectively. There was no antifungal activity. Another lectin was isolated from a different cultivar of soybean called little black soybean. The lectin was essentially similar to small glossy black soybean lectin except for a larger subunit molecular mass (31 kDa), a more potent mitogenic activity and lower thermostability. The results indicate that different cultivars of soybean produce lectins that are not identical in every aspect.

Keywords purification; melibiose-binding lectin; Chinese black soybean

Lectins are proteins that exhibit carbohydrate-binding specificity. They can be divided into groups, such as mannose-binding, glucose/mannose-binding, N-acetylgalactosamine-binding, galactose-binding, N-acetylgalactosamine-binding, fucose-binding, and sialic acid-binding, according to their carbohydrate-binding specificity. Lectins have been isolated from a diversity of organisms including flowering plants [1], animals [2,3], fungi [4], and bacteria [5]. Many biological activities, including anti-proliferative, anti-tumor, immunopotentiating, anti-insect, antifungal, antiviral and antibacterial activities, have been found in lectins [6−11]. As a result, lectins have captured the attention of many investigators.

Soybean is an agricultural crop of great importance. From it, many important proteins and non-proteinaceous compounds have been isolated. They comprise trypsin inhibitors [12], lectins [13], antifungal proteins [14], and phytoestrogens [15]. Phytoestrogens are reported to be protective against osteoporosis and cardiovascular disease [15,16]. Soybean trypsin inhibitor has an anti-tumor action [17]. Soybeans have been made into a variety of food products including drinks and bean curds. As stated in *Ben Cao Gang Mu* by Li Shizhen published in 1590, the black soybean is used in traditional Chinese medicine for treating backaches, combating debility and preventing aging. According to current popular literature, the black soybean has more protein, potassium and vitamins than the yellow soybean [18]. The former has chlorophyll and black pigment while the latter has carotenoids.

Different cultivars of the same species may produce different proteins; for example, the miraculin-like antifungal protein sativin and the ribosome-inactivating protein pisavin, which has similar sequence to miraculin, are produced by different cultivars of *Pisum sativum*, the sugar snap and the garden pea, respectively [19,20]. Different cultivars of soybean exist, including the yellow soybean, Chinese black soybean and Japanese black soybean. The yellow soybean produces trypsin inhibitors of the Bowman-Birk type and also the Kunitz type [12]. The Chinese dull black soybean produces only the Kunitz-type trypsin inhibitor [21], whereas the Japanese black soybean produces only the Bowman-Birk type [22]. Thus different cultivars may have dissimilar protein products.
Lectin from yellow soybeans has been isolated and characterized. However, it is not known if the Chinese black soybean produces the same lectin. The Chinese black soybean is a special cultivar of soybean that differs from the common yellow soybean in pharmacological activities in traditional Chinese medicine. Thus, it was deemed worthwhile to isolate a lectin from each of the two cultivars of Chinese black soybean, namely the Chinese small glossy black soybean and the Chinese little black soybean, to compare them with the lectin from common yellow soybean, and to explore other potentially exploitable activities of the lectins.

**Materials and Methods**

**Materials**

Small glossy black soybeans and little black soybeans (*Glycine max*) from China were purchased from a local vendor. They were authenticated by Prof. Shiuying Hu, Honorary Professor of Chinese Medicine, the Chinese University of Hong Kong (Hong Kong, China).

**Protein purification**

The same isolation procedure and assay were employed for both cultivars. The beans were homogenized in distilled water. Tris-HCl buffer (pH 7.4) was added to the dialyzed supernatant until the concentration of Tris attained 10 mM. The supernatant was then loaded on a 5 cm×20 cm column of Q Sepharose (GE Healthcare, Hong Kong, China) in 10 mM Tris-HCl buffer (pH 7.4). After removing the unadsorbed fraction, adsorbed fractions (Q1, Q2 and Q3) were eluted with starting buffer containing 0.2, 0.5 and 1 M NaCl, respectively. After examining the hemagglutinating effect of fractions Q1−3, the lectin-enriched fraction Q1 was dialyzed against 10 mM Tris-HCl buffer (pH 7.4). After removing the unadsorbed fraction, adsorbed fractions (Q1, Q2 and Q3) were eluted with starting buffer containing 0.2, 0.5 and 1 M NaCl, respectively. After examining the hemagglutinating effect of fractions Q1−3, the lectin-enriched fraction Q1 was dialyzed against 10 mM NH4OAc buffer (pH 4.5) at 4 ºC, and then chromatographed on a 2.5 cm×20 cm column of SP Sepharose (GE Healthcare), which had previously been equilibrated with and was then eluted with 10 mM NH4OAc buffer (pH 4.5). Unadsorbed proteins were eluted with the buffer, and then adsorbed proteins were eluted stepwise with the same buffer containing 0.2 M NaCl and 1 M NaCl, respectively. The fraction eluted with 0.2 M NaCl was then applied on a 1 ml Mono Q (GE Healthcare) column in 10 mM NH4HCO3 buffer (pH 9). Following elution of unadsorbed materials, adsorbed proteins (fraction S1, S2 and S3), were desorbed with two NaCl concentration gradients (0–0.3 M and adsorbed proteins 0.3–1 M) in 10 mM NH4HCO3 buffer (pH 9). The adsorbed fraction (S2), which was eluted by approximately 0.2 M NaCl, was the lectin-enriched fraction. It was dialyzed, lyophilized and subjected to gel filtration on a Superdex 75 HR 10/30 column (GE Healthcare) using an AKTA Purifier (GE Healthcare) in 10 mM NH4HCO3 buffer (pH 9.4). The first eluted absorbance peak constituted purified lectin.

**Molecular mass determination by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and by Fast Protein Liquid Chromatography (FPLC)-gel filtration**

SDS-PAGE was carried out in accordance with the procedure used by Nielsen and Reynolds [23], using a 12% resolving gel and a 5% stacking gel. The gel was cast on the miniplate Set II (Bio-Rad, Shanghai, China). Samples were diluted with sample loading buffer (10% glycerol, 0.4% SDS, 0.005% bromophenol blue and 20 mM EDTA in 0.5 M Tris-HCl, pH 7.5) followed by addition of 5% β-mercaptoethanol. The samples were boiled in a water bath for 10 min. Electrophoresis was performed at a constant current of 10 mA at room temperature. At the end of electrophoresis, the gel was stained with Coomassie brilliant blue. FPLC-gel filtration was carried out using a Superdex 200 HR 10/30 column (GE Healthcare) that had been calibrated with molecular-mass standards (GE Healthcare).

**Analysis of the N-terminal amino acid sequence**

Amino acid sequence analysis was carried out using an HP G1000A Edman degradation unit and an HP 1000 HPLC system from Hewlett-Packard (Palo Alto, USA)[20]

**Assay of hemagglutinating activity**

In the assay for lectin (hemagglutinating) activity, a serial two-fold dilutions of the lectin solution in microtiter U-plates (50 µl) were mixed with 50 µl of 2% suspension of rabbit red blood cells in phosphate-buffered saline (pH 7.2) at 20 ºC. The results were recorded after about 1 h, when the blank containing only red cells had fully sedimented and appeared as a dot at the bottom of the well. The hemagglutination titer, defined as the reciprocal of the highest dilution exhibiting hemagglutination, is defined as one hemagglutination unit. Specific activity is the number of hemagglutination units per mg protein [11].

**Inhibition of lectin-induced hemagglutination by carbohydrates**

The hemagglutinating inhibition tests to investigate inhibition of lectin-induced hemagglutination by various carbohydrates for 30 min at room temperature were performed in a manner analogous to the hemagglutination test [11]. Serial two-fold dilutions of sugar samples were prepared.
in phosphate buffered saline. All of the dilutions were mixed with an equal volume (25 µl) of a solution of the lectin with 32 hemagglutination units. The mixture was allowed to stand for 30 min at room temperature, and then mixed with 50 µl of 2% rabbit erythrocyte suspension. The minimum concentration of the sugar in the final reaction mixture, which completely inhibited 32 hemagglutination units of the lectin preparation, was calculated.

**Effect of temperature and pH on lectin-induced hemagglutination**

The effects of temperature and pH on hemagglutinating activity of the lectin were examined [11]. A solution of lectin was incubated at various temperatures (0, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 °C) for 30 min. The tubes were then put on ice and assay of hemagglutinating activity was then carried out. A solution of lectin was incubated at various pH values (pH 1–14) for 30 min. The reaction mixtures in the tubes were neutralized and assay of hemagglutinating activity was then carried out.

**Assay of mitogenic activity**

Incubation of splenocytes from BALB/c mice (20–25 g) was carried out at 37 °C in a humidified atmosphere of 5% CO₂ in the presence or absence of the lectin for 24 h in a 96-well culture plate. We added 10 µl (methyl-³H) thymidine (0.25 µCi; GE Healthcare) before the splenocytes were incubated for another 6 h under the same conditions. The splenocytes were then harvested onto a glass fiber filter, and the radioactivity was measured [24–26].

**Assay of antiproliferative activity on tumor cell lines**

Tumor cells, MCF7 and HepG2, in their exponential growth phase were seeded into a culture plate and incubated for 3 h before addition of the lectin. Incubation was carried out for another 48 h. Radioactive precursor, 1 µCi of (methyl-³H)thymidine, was then added to each well and incubated for 6 h. The cultures were then harvested, and the incorporated radioactivity was determined [25].

**Assay of HIV reverse transcriptase inhibitory activity**

The assay was carried out by using an enzyme-linked immunosorbent assay kit as described by Collins *et al* [16]. The assay for HIV reverse transcriptase inhibitory activity was carried out in accordance with instructions supplied with the assay kit from Boehringer Mannheim (Berlin, Germany) since some antifungal proteins possess this activity. The assay takes advantage of the ability of reverse transcriptase to synthesize DNA, starting from the template/primer hybrid poly(A) oligo(dT)₁₅. The digoxigenin- and biotin-labeled nucleotides in an optimized ratio are incorporated into one of the same DNA molecule, which is freshly synthesized by the reverse transcriptase (RT). The detection and quantification of synthesized DNA as a parameter for RT activity follows a Sandwich ELISA protocol. Biotin-labeled DNA binds to the surface of microtiter plate modules that have been pre-coated with streptavidin. In the next step, an antibody to digoxigenin, conjugated to peroxidase, binds to the digoxigenin-labeled DNA. In the final step, the peroxidase substrate is added. The peroxidase enzyme catalyzes the cleavage of the substrate, producing a colored reaction product. The absorbance of the sample at 405 nm can be determined using a microtiter plate reader and is directly correlated to the level of RT activity. A fixed amount (4–6 ng) of recombinant HIV-1 reverse transcriptase was used. The inhibitory activity of the lectin was calculated as percent inhibition as compared to a control without the lectin [27].

**Assay of HIV-I integrase and Severe acute respiratory syndrome (SARS) proteinase activities**

The assay of HIV-I integrase and Severe acute respiratory syndrome (SARS) proteinase activities was conducted as previously reported [28].

*Expression and purification of recombinant HIV-I integrase*  The plasmid that expressed His-tagged wild-type HIV-1 integrase, pT7-7-His (Y|TX)-HIV-1-IN, was a generous gift from Professor S. A. Chow (School of Medicine, UCLA). To express the protein, a 1-liter culture of *E. coli* BL21(DE3) cells containing the expression plasmid was grown at 37 °C until OD₆₀₀ reached 0.7–0.8. Cells were induced by addition of 0.8 mM isopropyl-β-D-thiogalactopyranoside (IPTG) and harvested, after 4 h of incubation, by centrifugation at 6000 g for 10 min at 4 °C. Cells were suspended at a concentration of 10 ml/g wet cell paste in 20 mM Tris-HCl buffer (pH 8.0) containing 0.1 mM EDTA, 2 mM β-mercaptoethanol, 0.5 M NaCl and 5 mM imidazole. Lysozyme was added to a concentration of 0.2 mg/ml. After incubation at 4 °C for 1 h, the lysate was sonicated and centrifuged at 40,000 g at 4 °C for 20 min. The pellet was homogenized in 50 ml buffer A (20 mM Tris-HCl, pH 8.0, 2 M NaCl, 2 mM β-mercaptoethanol) containing 5 mM imidazole. The suspension was rotated at 4 °C for 1 h, and cleared by centrifugation at 40,000 g at 4 °C for 20 min. The supernatant was loaded onto a 1-ml chelating Sepharose
Isolation of small glossy black soybean lectin and little black soybean lectin

The crude extract of small glossy black soybeans was fractionated on a Q Sepharose column into an unadsorbed fraction and three adsorbed fractions (Q1, Q2 and Q3) were obtained by stepwise elution with 0.2, 0.5 and 1 M NaCl in Tris-HCl buffer [Fig. 1(A)]. Hemagglutinating activity was confined to fraction Q1. This fraction was resolved on an SP Sepharose column into an unadsorbed fraction and two adsorbed fractions (SP1 and SP2) respectively when eluted with 0.2 M NaCl and 1 M NaCl in the NH$_4$OAc buffer [Fig. 1(B)]. Hemagglutinating activity resided in the smaller fraction SP1. Fraction SP1 was further resolved by ion exchange chromatography on an FPLC-Mono Q column into a tiny unadsorbed fraction and several adsorbed fractions [Fig. 1(C)]. Hemagglutinating activity was recovered only in fraction S2, which was subsequently separated on a Superdex 75 column into a sharp larger fraction and a smaller fraction. Activity was detected only in the larger fraction [Fig. 1(D)].

From Chinese little black soybeans, a lectin was isolated using the same procedure except for the omission of ion exchange chromatography on Mono Q (Fig. 2).

Determination of molecular weight

The small glossy black soybean lectin product appeared as a single band with a molecular mass of 25 kDa in SDS-PAGE (Fig. 3), and exhibited a single peak with a molecular mass of 50.2 kDa in gel filtration on Superdex 75 [Fig. 1(D)].

The little black soybean lectin appeared as a single band with a molecular mass of 31 kDa in SDS-PAGE (Fig. 3), and exhibited a single peak with a molecular mass of 62 kDa in gel filtration on Superdex 75 [Fig. 2(C)].

Determination of N-terminal sequence

The small glossy black soybean lectin was obtained with a specific activity of 7168 U/mg and a purification fold of 29 (Table 1). The N-terminal sequence of the lectin was 'AETVFSWNKVF PKQ$^{15}$, which was identical to lectin from yellow soybean and similar to partial sequences near the N-terminals of Medicago sativa$^{(7)}$ and Robinia pseudoacacia$^{(8)}$ lectins ('EVSF SFTKFVP$^{8}$'). There was only one peak in every sequencing cycle, indicating that the lectin preparation was homogeneous. It appears that soybean lectins are similar in N-terminal sequence to only some members of the pea family Fabaceae.

The N-terminal sequence of little black soybean lectin...
Lectin from Chinese black soybean

Fig. 1 Purification of small glossy black soybean lectin by chromatography

(A) The black soybean extract was applied on a Q Sepharose column (5 cm × 20 cm). After elution of unadsorbed proteins with 10 mM Tris-HCl buffer (pH 7.4), the column was eluted stepwise with 0.2 M NaCl, 0.5 M NaCl, and 1 M NaCl added to the Tris-HCl buffer as indicated by the arrows. (B) Fraction Q1 from the Q Sepharose column was dialyzed and applied on an SP Sepharose column (2.5 cm × 20 cm) in 10 mM NH₄OAc buffer (pH 4.5). After elution of unadsorbed proteins, the column was eluted stepwise with 0.2 M NaCl and then with 1 M NaCl added to the buffer as indicated by the arrows. (C) Fraction SP1 from the SP Sepharose column was loaded on a 1 ml Mono Q column. Following elution of unadsorbed proteins with 10 mM NH₄HCO₃ buffer (pH 9), adsorbed proteins were eluted sequentially, first with a 0–0.3 M NaCl gradient and then with a 0.3–1 M NaCl gradient. (D) Fraction S2 from the Mono Q column was subjected to gel filtration on a Superdex 75 HR 10/30 column in 10 mM NH₄HCO₃ buffer (pH 9.4).

Fig. 2 Purification of little black soybean lectin by chromatography

(A) Little black soybean extract was applied on a Q Sepharose column (5 cm × 20 cm). After elution of unadsorbed proteins with 10 mM Tris-HCl buffer (pH 7.4), the column was eluted stepwise with 0.2 M NaCl, 0.5 M NaCl, and 1 M NaCl added to the Tris-HCl buffer as indicated by the arrows. (B) Fraction Q1 from the Q Sepharose column was dialyzed and applied on an SP Sepharose column (2.5 cm × 20 cm) in 10 mM NH₄OAc buffer (pH 4.5). After elution of unadsorbed proteins, adsorbed proteins were eluted with a linear NaCl concentration gradient (0–0.5 M) in 100 mM NH₄OAc buffer (pH 4.5) as indicated by the slanting line across the chromatogram. Lectin activity was observed only in fraction SP2. (C) Fraction SP2 from the SP Sepharose column was subjected to gel filtration on a Superdex 75 HR 10/30 column in 10 mM NH₄HCO₃ buffer (pH 9.4).
was identical to that of small glossy black soybean lectin (Table 2).

**Determination of sugar specificity, pH stability and thermostability of hemagglutinating activity**

Melibiose, arabinose, raffinose and galactose at 40 mM concentration inhibited the hemagglutinating activity of the small glossy black soybean lectin. However, glucose, fucose, rhamnose, mannose, lactose, xylose, glucuronic acid, polygalacturonic acid, and mannosamine had no effect (Table 2). The hemagglutinating activity was stable after exposure for 30 min to the pH range 3–12 and the temperature range 0–70 °C.

The carbohydrate specificity of little black soybean lectin was identical to that of small glossy black soybean lectin. The hemagglutinating activity of little black soybean lectin was stable only up to 40 °C for 20 min, but the small glossy black soybean lectin was stable up to 70 °C for 30 min.

**Other biological activities**

The small glossy black soybean lectin inhibited proliferation of HepG2 cells and MCF7 cells with an IC₅₀ of 4.1 µM and 2.6 µM, respectively (Fig. 4) and the activity of HIV-1 reverse transcriptase with an IC₅₀ of 2.82 µM (Fig. 5).
Table 2 Comparison of sugar specificity of lectins from different cultivars of soybean

| Sugar (40 mM)          | Yellow soybean lectin | Chinese small glossy black soybean lectin | Chinese little black soybean lectin |
|------------------------|-----------------------|------------------------------------------|-------------------------------------|
| Melibiose              | –                     | +                                        | +                                   |
| Galactose              | –                     | +                                        | +                                   |
| Arabinose              | +                     | +                                        | +                                   |
| Raffinose              | +                     | –                                        | –                                   |
| N-acetylgalactosamine  | –                     | –                                        | –                                   |

Glucose, fucose, rhamnose, mannose, lactose, xylose, glucuronic acid, polygalacturonic acid, and mannosamine were all inactive when tested in 500 mM. +, inhibition of hemagglutinating activity of lectin; −, no inhibition of hemagglutinating activity of lectin.

Fig. 4 Antiproliferative activity of small glossy black soybean lectin on (A) HepG2 hepatoma cells and (B) MCF7 breast cancer cells. These cells were treated with the lectin for 72 h at 37 °C in an atmosphere of 95% CO₂ and 5% O₂. Results are presented as the mean±SD (n=3). IC₅₀ values for HepG2 cells and MCF cells were 4.1 μM and 2.6 μM, respectively.

Fig. 5 HIV-1 reverse transcriptase inhibition by small glossy black soybean lectin and little black soybean lectin. Results are presented as the mean±SD (n=3). IC₅₀ of small glossy black soybean was 2.82 μM. IC₅₀ of little black soybean lectin was 3.54 μM.

The mitogenic activity of the lectin on mouse splenocytes is shown in Fig. 6. Both the lectin and Con A stimulated maximal mitogenic response at similar concentrations. However, the maximal response achieved by small glossy black soybean lectin was weaker than that evoked by Con A (Fig. 6). Small glossy black soybean lectin did not inhibit HIV integrase or SARS proteinase when tested up to 20 μM (data not shown).

The little black soybean lectin inhibited HIV-1 reverse transcriptase with an IC₅₀ of 3.54 μM (Fig. 5). The maximal mitogenic response that both the small glossy black soybean lectin and the little black soybean lectin elicited was about 20% of the maximal response to Con A. The maximal mitogenic response was achieved by similar concentrations of small glossy black soybean lectin, little black soybean lectin and Con A (Fig. 6).
Lectin from Chinese black soybean

Comparison of two black soybean lectins

A comparison of the characteristics of the two black soybean lectins isolated in this study with those of soybean lectins is presented in Table 3.

Discussion

The two Chinese small glossy black soybean lectins differ somewhat from common yellow soybean lectin (hereinafter referred to as soybean lectin) in carbohydrate specificity. Soybean lectin is galactose-specific [13], whereas Chinese black soybean lectins are melibiose-specific although the latter two are also galactose-specific. The two Chinese black soybean lectins potently inhibit HIV-1 reverse transcriptase (IC50 about 2.8 µM). It is known that some lectins exhibit HIV-1 reverse transcriptase inhibitory activity and inhibit HIV replication [27, 29, 30]. However, just like the French bean defensin [28], the two black soybean lectins are devoid of HIV-1 integrase inhibitory and SARS proteinase inhibitory activity. The antiproliferative and anti-tumor activities of lectins are well documented [25, 27, 31–33]. Chinese small glossy black soybean lectin exerts potent antiproliferative activity toward HepG2 and MCF7 cells, with an IC50 of 4.1 µM and 2.6 µM, respectively. Both black soybean lectins manifest weaker mitogenic activity than Con A toward splenocytes. This is in line with findings on other lectins [24, 25].

The mitogenic activity of soybean lectin toward human and murine lymphocytes is enhanced after polymerization by physical or chemical means [34]. Soybean lectin has immunomodulatory and anti-tumor actions [34]. Natural suppressor cells from the spleen and cyclophosphamide-generated suppressor cells react specifically with soybean lectin and can thus be isolated by agglutination from the bone marrow [35, 36]. Soybean lectin is employed for purging the marrow of T cells during treatment of acute lymphoblastic leukemia in order to decrease the risk of graft-versus-host disease [37]; soybean lectin reacts with and eliminates cancer cells from the marrow [38]. This investigation’s findings on the two black soybean lectins’ mitogenic activity towards splenocytes and their antiproliferative activity toward tumor cells are consistent with previous observations [34, 38]. An anti-tumor action mechanism of soybean lectins has been proposed involving the action of the lectins on tumor cell membranes, the reduction of tumor cell proliferation, the induction of tumor-specific cytotoxicity of macrophages, and apoptosis. Thus, tumor cells are more susceptible to attack by macrophages after treatment with lectins. Furthermore, lectins exert an immunomodulatory effect on altering interleukins production [39].

Soybean lectin reportedly reacts preferentially with some rumen fungi [40], but an antifungal action has not been demonstrated. Likewise, the two black soybean lectins are devoid of antifungal activity (data not shown).

| Molecular weight (kDa) | Number of subunit | pH stability (pH) | Thermostability | Anti-tumor activity | Mitogenic activity |
|------------------------|-------------------|-------------------|-----------------|--------------------|--------------------|
| **Yellow soybean lectin** | 122 | 4 | pH 2–13 | Up to 50 ºC | Existing | Existing |
| **Chinese small glossy black soybean lectin** | 50 | 2 | pH 2–13 | Up to 70 ºC | HepG2 IC50: 4.1 µM; MCF7 IC50: 2.6 µM | Maximal response about 20% of Con A |
| **Chinese little black soybean lectin** | 62 | 2 | pH 3–13 | Up to 40 ºC | Existing | Maximal response about 20% of Con A |
In fact, to date, only a handful of lectins have been reported with antifungal activity [41,42]. Small glossy black soybean lectin has fair pH stability and thermostability. Its hemagglutinating activity is preserved in the pH range 3–12 and in the temperature range 0–70 ºC. Little black soybean lectin is stable in the pH range 3–12 and in the temperature range 0–40 ºC. Yellow soybean lectin is active in the pH range 3–12 and in the temperature range 0–50 ºC. French bean lectin is stable in pH range 4–10 and in the temperature range 0–90 ºC [30]. Hence the French bean lectin and small glossy black soybean lectin appear to be similar in stability.

The protocol employed in the present investigation for purifying the two black soybean lectins entailed successive ion exchange chromatography on Q Sepharose, SP Sepharose and Mono Q (with the exception of little black soybean lectin), and gel filtration on Superdex 75. French bean lectin was isolated using a similar procedure involving chromatography on SP Sepharose, Affi-gel blue gel, Q Sepharose and Superdex 200 [28]. The two black soybean lectins were adsorbed on SP Sepharose and Q Sepharose. Thus, the three leguminous lectins appear to have similar chromatographic behavior. A 29-fold purification was obtained in the present study for small glossy black soybean lectin compared to 10-fold purification in the case of French bean lectin [28]. Thus lectins from the two cultivars of Chinese black soybean appear to differ in subunit molecular mass, thermostability and mitogenic activity, although they share the same N-terminal amino acid sequence and carbohydrate specificity and have similar HIV-reverse transcriptase inhibitory activity. This finding is reminiscent of the observation that lectins from different cultivars of the bean Phaseolus vulgaris, such as the pinto bean [24], haricot bean [25], red kidney bean [30], and dark red kidney bean [26], have similar but not identical N-terminal sequences. Their other characteristics, including carbohydrate specificity and antiproliferative activity toward tumor cells, may not be all alike [24–26,30].

The results of the present investigation indicate that different soybean cultivars produce lectins that are not identical. The lectins isolated in the present study are characterized by potent antiproliferative activity toward cancer cells and inhibitory activity against HIV-1 reverse transcriptase, as well as moderate pH stability and thermostability. Its antiproliferative activity against cancer cells of mammalian origin and HIV-1 reverse transcriptase inhibitory activities indicate that it is a defense protein. This is in line with the defense function of lectins in plants [43]. As such, the biological activities of black soybean lectins are potentially exploitable in medicine.

References
1 Kang TB, Song SK, Yoon TJ, Yoo YC, Lee KH, Her E, Kim JB. Isolation and characterization of two Korean mistletoe lectins. J Biochem Mol Biol 2007, 40: 959–965
2 Watanabe Y, Shinya N, Shinozaki F, Yokoyama H, Kominami J, Nakamura-Tsurtu S, Hirabayashi J et al. Isolation and characterization of l-rhamnose-binding lectin, which binds to microsporidian Glugea plecoglossi, from ayu (Plecoglossus altivelis) eggs. Dev Comp Immunol 2008, 32: 487–494
3 Watanabe T, Matsuoi I, Maruyama J, Kitamoto K, Ito Y. Identification and characterization of an intracellular lectin, calnexin, from Aspergillus oryzae using N-glycan-conjugated beads. Biosci Biotechnol Biochem 2007, 71: 2688–2696
4 Lameignere E, Malinovska L, Slavikova M, Duchaud E, Mitchell EP, Varro T, Sedo O et al. Structural basis for mannose recognition by a lectin from opportunistic bacteria Burkholderia cenocepacia. Biochem J 2008, 411: 307–318
5 Kaur M, Singh K, Rup PJ, Kamboj SS, Saxena AK, Sharma M, Bhagat M et al. A tuber lectin from Arisaema jacquemontii Blume with anti-insect and anti-proliferative properties. J Biochem Mol Biol 2006, 39: 432–440
6 Oliveira MD, Andrade CA, Santos-Magalhães NS, Coelho LC, Teixeira JA, Carneiro-da-Cunha MG, Correia MT. Purification of a lectin from Eugenia uniflora L. seeds and its potential antibacterial activity. Lett Appl Microbiol 2008, 46: 371–376
7 Abdullaev FI, de Mejia EG. Antitumor effect of plant lectins. Nat Toxins 1997, 5: 157–163
8 Rubinstein N, Ilarregui JM, Toscano MA, Rabichovich GA. The role of galectins in the initiation, amplification and resolution of the inflammatory response. Tissue Antigens 2004, 64: 1–12
9 Herre J, Willment JA, Gordon S, Brown GD. The role of Dectin-1 in antifungal immunity. Crit Rev Immunol 2004, 24: 193–203
10 Macedo ML, Damico DC, Freire MG, Toyama MH, Marangoni S, Novello JC. Purification and characterization of an N-acetylgalactosamine-binding lectin from Koelreuteria paniculata seeds and its effect on the larval development of Callosobruchus maculatus (Coleoptera: Bruchidae) and Anagasta kuehniella (Lepidoptera: Pyralidae). J Agric Food Chem 2003, 51: 2980–2986
11 Wong JH, Ng TB. Isolation and characterization of a glucose/mannose-specific lectin with stimulatory effect on nitric oxide production by macrophages from the emperor banana. Int J Biochem Cell Biol 2006, 38: 234–243
12 Losso JN. The biochemical and functional food properties of the Bowman-Birk inhibitor. Crit Rev Food Sci Nutr 2008, 48: 94–118
13 Gordon JA, Blumberg S, Lis H, Sharon N. Purification of soybean agglutinin by affinity chromatography on sepharose-N-epsilon-acetylglucosamine-binding beads. Biosci Biotechnol Biochem 2008, 40: 243–246
14 Graham JS, Burkhart W, Xiong J, Gillikin JW. Complete amino acid sequence of soybean leaf P21: similarity to the thaumatinin-like polypeptides. Plant Physiol 1992 98: 163–165
15 Vatanparast H, Chilibeck PD. Does the effect of soy phytoestrogens on bone in postmenopausal women depend on the equal-producing phenotype? Nutr Rev 2007, 65: 294–299
16 Jackman KA, Woodman OL, Soby CG. Isoflavones, equol and cardiovascular disease: Pharmacological and therapeutic insights.
Lectin from Chinese black soybean

Acta Biochim Biophys Sin (2008) | Volume 40 | Issue 12 | Page 1038

17 Kobayashi H, S. Fuku Y, Yoshida R, Kanada Y, Nishiyama S, Suzuki M, Kanayama N et al. Suppressing effects of dietary suplementation of soybean trypsin inhibitor on spontaneous, experimental and peritoneal disseminated metastasis in mouse model. Int J Cancer 2004, 112: 519–524

18 Jiangsu New Medical School. The Great Dictionary of Chinese Medicine. Shanghai Peoples’ Press 1977, 2: 2382–2383

19 Ye XY, Wang HX, Ng TB. Sativin: a novel antifungal miraculin-like protein isolated from legumes of the sugar snap Pisum sativum var. macrocarpon. Life Sci 2000, 67: 775–781

20 Lam SSL, Wang HX, Ng TB. Purification and characterization of novel ribosome inactivating proteins, alpha- and beta-pisavins, from seeds of the garden pea Pisum sativum. Biochim Biophys Res Commun 1998, 253: 135–142

21 Lin, P, Ng TB. A stable trypsin inhibitor from Chinese dull black soybean with potentially exploitable activities. Process Biochem 2008, 43: 992–998

22 Ho VS, Ng TB. A Bowman-Birk trypsin inhibitor with antiproliferative activity from Hokkaido large black soybeans. J Pept Sci 2008, 14: 278–282

23 Nielsen TB, Reynolds JA. Measurements of molecular weights by gel electrophoresis. Methods Enzymol 1978, 48: 3–10

24 Wong JH, Wong CC, Ng TB. Purification and characterization of a galactose-specific lectin with mitogenic activity from pinto beans. Biochim Biophys Acta 2006, 1760: 808–813

25 Wong JH, 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

26 Xia L, Ng TB. A hemagglutinin with mitogenic activity from dark red kidney beans. J Chromatogr B Analyt Technol Biomed Life Sci 2006, 844: 213–216

27 Wong JH, Ng TB. Purification of a trypsin-stable lectin with antiproliferative and HIV-1 reverse transcriptase inhibitory activity. Biochim Biophys Res Commun 2003, 301: 545–550

28 Ye XY, Ng TB, Tsang PW, Wang J. Isolation of a homodimeric lectin with antifungal and antiviral activities from red kidney bean (Phaseolus vulgaris) seeds. J Protein Chem 2001, 20: 367–375

29 Sato Y, Okuyama S, Hori K. Primary structure and carbohydrate binding specificity of a potent anti-HIV lectin isolated from the filamentous cyanobacterium Oscillatoria agardhii. J Biol Chem 2007, 282: 11021–11029

30 Leung EH, Wong JH, Ng TB. Concurrent purification of two defense proteins from French bean seeds: a defensin-like antifungal peptide and a hemagglutinin. J Pept Sci 2008, 14: 349–353

31 Kobayashi H, Suzuki M, Kanayama N, Terao T. A soybean Kunitz trypsin inhibitor suppresses ovarian cancer cell invasion by blocking urokinase up-regulation. Clin Exp Metastasis 2004, 21: 159–166

32 Choi SH, Lyu SY, Park WB. Mistletoe lectin induces apoptosis and telomerization in human A253 cancer cells through dephosphorylation of Akt. Arch Pharm Res 2004, 27: 68–76

33 Ganguly C, Das S. Plant lectins as inhibitors of tumor growth and modulators of host immune response. Chemotherapy 1994, 40: 272–278

34 Lotan R, Lis H, Rosenwasser A, Novogrodsky A, Sharon N. Enhancement of the biological activities of soybean agglutinin by cross-linking with glutaraldehyde. Biochem Biophys Res Commun 1973, 55: 1347–1355

35 Hoskin DW, Bowser DA, Brooks-Kaiser JC. Soybean agglutinin-positive natural suppressor cells in mouse bone marrow inhibit interleukin 2 production and utilization in mixed lymphocyte reactions. J Leukoc Biol 1992, 51: 649–656

36 Brooks-Kaiser JC, Bourque LA, Hoskin DW. Heterogeneity of splenic natural suppressor cells induced in mice by treatment with cyclophosphamide. Immunopharmacology 1993, 25: 117–129

37 Reisner Y, Kapoor N, Kirkpatrick D, Pollack MS, Dupont B, Good RA, O’Reilly RJ. Transplantation for acute leukemia with HLA-A and B nonidentical parental marrow cells fractionated with soybean agglutinin and sheep red blood cells. Lancet 1981, 2: 327–331

38 Reisner Y. Differential agglutination by soybean agglutinin of human leukemia and neuroblasto cell lines: potential application to autologous bone marrow transplantation. Proc Natl Acad Sci U S A 1983, 80: 6657–6661

39 de Mejia EG, Bradford T, Hasler C. The anticarcinogenic potential of soybean lectin and lanusin. Nutr Rev 2003, 61: 239–246

40 Baintner K, Farningham DA, Bruce LA, MacRae JC, Pusztai A. Fate of the antinutritive proteins of soyabean in the ovine gut. Zentralbl Veterinarmed A 1993, 40: 427–431

41 Gozia O, Ciopraga J, Bentia T, Lunga M, Zamfirescu I, Tudor R, Roseanu A et al. Antifungal properties of lectin and new chitinases modulators of host immune response. Chemotherapy 1994, 40: 378–380

42 Verheyden R, Pleinckx J, Maes D, Peermans HA, Wynn I, Willem R, Martin J. “H NMR study of the interaction of N, N,N′,N′-triacetyl chitotriose with AC-AMP2, sugar binding antimicrobial protein isolated from Amaranthus caudatus. CR Acad Sci Ser III 1995, 316: 780–792

43 Peumans WJ, Van Damme EJ. Lectins as plant defense proteins. Plant Physiol 1995, 109: 347–352