ISSN 1672-9145 **Acta Biochimica et Biophysica Sinica** 2007, **39**(7): 507–519 **CN** 31-1940/Q

Purification and Characterization of a Mannose-binding Lectin from the Rhizomes of *Aspidistra elatior* Blume with Antiproliferative Activity

Xiaochao XU[1], Chuanfang WU[1], Chao LIU[2], Yongting LUO[1], Jian LI[1], Xinping ZHAO[1], Els Van DAMME[3], and Jinku BAO[1]*

[1] *College of Life Sciences, Sichuan University, Chengdu 610064, China;*
[2] *Leshan Teachers' College, Leshan 614000, China;*
[3] *Department of Molecular Biotechnology, Ghent University, 9000 Gent, Belgium*

Abstract A lectin with a novel N-terminal amino acid sequence was purified from the rhizomes of *Aspidistra elatior* Blume by ammonium sulphate precipitation, ion exchange chromatography on diethylaminoethyl-Sepharose and carboxymethyl-Sepharose and gel filtration chromatography on Sephacryl S-100. The *A. elatior* Blume lectin (AEL) is a heterotetramer with a molecular mass of 56 kDa and composed of two homodimers consisting of two different polypeptides of 13.5 kDa and 14.5 kDa held together by noncovalent interactions. Hapten inhibition assay indicated that hemagglutinating activity of AEL towards rabbit erythrocytes could be inhibited by *D*-mannose, mannan, thyroglobulin and ovomucoid. The lectin was stable up to 70 ºC, and showed maximum activity in a narrow pH range of 7.0–8.0. Chemical modification and spectrum analysis indicated that tryptophan, arginine, cysteine and carboxyl group residues were essential for its hemagglutinating activity. However, they might not be present in the active center, except some carboxyl group residues. AEL also showed significant *in vitro* antiproliferative activity towards Bre-04 (66%), Lu-04 (60%) and HepG2 (56%) of human cancer cell lines.

Keywords antiproliferative activity; *Aspidistra elatior* Blume; chemical modification; hemagglutinating activity; mannose-binding lectin

Lectins are carbohydrate-binding proteins or glycoproteins of non-immune origin capable of specific recognition of, and reversible binding to, carbohydrates without altering their covalent structure [1]. In addition to binding to carbohydrates, many plant lectins exhibit specific interactions with small molecules that are predominantly hydrophobic in nature. On the basis of recent advances in molecular structure and biological specificities, a new definition of lectins has been proposed as proteins possessing at least one non-catalytic domain, which binds reversibly to a specific monosaccharide or oligosaccharide [2]. Owing to the fine specificity, lectins have been used for various applications in biomedical sciences including cancer research. A variety of alterations in carbohydrate structure have been observed in cancer cells. These might involve increased sialylation, increased branching of complex carbohydrates, or occasionally emergence of some novel structures [3]. Lectins could serve as excellent probes to study these altered glycosylation patterns. A few lectins, including those from *Helix pomatia* [4] and *Agaricus bisporus* [5], are being investigated for their use in cancer research and therapy. As an important superfamily, monocot mannose-binding lectins have been isolated and characterized from various monocot families including Liliaceae, Alliaceae, Orchidaceae, Amaryllidaceae and Araceae [6]. To the best of our knowledge, earlier reported lectins of Liliaceae plants were isolated from the leaves and bulbs, such as aloe [7] and tulip lectins [8]. In recent

Received: March 11, 2007 Accepted: April 13, 2007
This work was supported by the grants from the National Natural Science Foundation of China (30000032 and 30270331), the Science and Technology Fund for Distinguished Young Scholars of Sichuan Province (06ZQ026-035), the Key Technologies R&D Program of Sichuan Province (2006Z08-010) and European Commission (EMPRO) and the Centers of Excellence of the K. U. Leuven (EF/05/15)
*Corresponding author: Tel, 86-28-85410672; Fax, 86-28-85417281; E-mail, baojinku@yahoo.com.cn

DOI: 10.1111/j.1745-7270.2007.00305.x

years, some lectins were also found in the rhizomes of Liliaceae plants, such as *Smilax glabra* agglutinin (SGA) [9], the second *S. glabra* agglutinin [10], *Paris quadrifolia* lectin [11], *Polygonatum multiflorum* lectin (PML) [12] and *Polygonatum cyrtonema* lectin (PCL) [13]. *Aspidistra elatior* Blume, a member of Liliaceae, is a traditional Chinese medicinal herb and ornamental plant species. Its rhizomes and leaves show diuresis, abirritation and detumescence effects on certain illnesses. For a long time it has been used in China to cure injuries from falls, as well as rheumatic fever and rheumatism. In recent years, research into its biochemical constituents was mainly focused on the steroidal compounds [14]. However, there is no report on the bioactive proteins from *A. elatior* Blume.

The present investigation focused on tracing for bioactive proteins from proteinaceous components in *A. elatior* Blume. A novel lectin (designated AEL) from the rhizomes of *A. elatior* Blume was purified and found to possess interesting activities, such as antiproliferative activity to human tumor cell lines Bre-04, Lu-04, HepG2 and Pro-01.

Materials and Methods

Plant material

The rhizomes of *A. elatior* Blume were collected from the campus of Sichuan University (Chengdu, China) in August.

Cell lines, chemicals and reagents

The human cancer cell lines Pro-01 (prostate), Lu-04 (lung), Bre-04 (breast), HepG2 (liver) and HeLa (cervix) were purchased from Di'ao Group (Chengdu, China). *N*-bromosuccinimide (NBS), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), [1-^{14}C]glycine ethyl ester, 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), p-nitrobenzene sulfonyl fluoride, diethyl pyrocarbonate and p-nitrophenylglyoxal were obtained from Serva (Heidelberg, Germany). Most monosaccharides, oligosaccharides, glycoproteins and 2-(N-morpholino) ethanesulfonic acid (MES) were purchased from Sigma (St. Louis, USA). Standard molecular weight markers and gel filtration markers were the products of Amersham (Uppsala, Sweden). All the other chemicals were of analytical grade.

Purification of agglutinin from rhizomes of *A. elatior*

Two hundred grams of *A. elatior* Blume rhizomes were crushed and soaked in 500 ml of 0.145 M NaCl, and the suspension was stirred for 24 h at 4 ºC and filtered through muslin cloth. The filtrate was centrifuged at 7000 *g* for 30 min and the supernatant was precipitated at 80% ammonium sulphate saturation. The precipitates were dissolved in 20 mM Tris-HCl (pH 8.0) and extensively dialyzed against the same buffer. The dialysate was centrifuged (6000 *g* for 20 min) and the clear supernatant was loaded on a diethylaminoethyl-Sepharose column (2 cm×20 cm). The unadsorbed protein (D1) was eluted with equilibrating buffer (Tris-HCl, 20 mM, pH 8.0) until the absorbance at 280 nm was negligible. Then the adsorbed proteins were eluted with a linear 0–0.5 M NaCl gradient. The second adsorbed fraction (D3) showing hemagglutinating activity was pooled, dialyzed against sodium acetate buffer (20 mM, pH 4.6) and loaded on a carboxymethyl-Sepharose column (2 cm×20 cm) which had previously been equilibrated with the same buffer. After elution of the unadsorbed fraction C1, the column was eluted stepwise with 100 mM, 250 mM and 400 mM NaCl in the starting buffer. Fractions (C4 and C5) with hemagglutinating activity were collected, concentrated to approximately 4 mg/ml and loaded on the Sephacryl S-100 column (2 cm×100 cm). The peak fraction (S2) showing hemagglutinating activity was pooled, extensively dialyzed against deionized water, lyophilized and stored at –20 ºC for further use.

Homogeneity and molecular weight determination

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out using 15% (*W*/*V*) acrylamide in gels as described by Laemmli and Favre [15]. Protein bands were visualized by Coomassie Brilliant Blue R-250. Native molecular mass of the lectin was determined by gel filtration chromatography on the same Sephacryl S-100 column, previously equilibrated with 10 mM phosphate-buffered saline (PBS; pH 7.2), according to the method of Whitakar [16] and Andrews [17]. The protein was eluted (0.2 ml/min) with the same buffer. Molecular standards such as bovine serum albumin (BSA; 66 kDa), ovalbumin (45 kDa), lactoglobulin (36 kDa) and carbonic anhydrase (30 kDa) from Amersham were used to calibrate the column.

Protein determination

Protein concentration was determined according to Lowry *et al.* [18] using BSA as a standard. Total neutral sugar was determined using the phenol/sulphuric method [19], with *D*-glucose as a standard.

Hemagglutination and hapten inhibition assays

After adding 25 μl of serial 2-fold dilution of the lectin into 96-well microtiter U-plates, an equal volume of 2%

(*V*/*V*) suspension of rabbit erythrocytes (3×10^8 cells/ml) was added [8]. Agglutination was assessed after 1 h at room temperature when the blank had fully sedimented. Agglutination activity was expressed as the reciprocal of the highest dilution that gave a positive result and was reckoned as one hemagglutination unit. To find the carbohydrate specificity of AEL, sugar inhibition assay was carried out in a manner analogous to the hemagglutination test. Serial 2-fold dilutions of sugar samples were prepared in PBS (10 mM, pH 7.2). All of the dilutions were mixed with an equal volume (25 μl) of a solution of the agglutinin with four hemagglutination units. Sugars or their derivatives were tested at a concentration of 100 mM, polysaccharides and glycoproteins were tested at a concentration of 4 mg/ml. The minimum concentration of the sugar in the final mixture that completely inhibited the lectin-induced hemagglutination was taken as the minimal inhibitory sugar concentration.

Effect of temperature, pH and denaturants on the hemagglutinating activity of AEL

To examine the thermostability, lectin solution (1 mg/ml) prepared in PBS (10 mM, pH 7.2) was incubated at different temperatures ranging from 30 ºC to 100 ºC for 30 min, with 10 ºC increases at each step until 100 ºC. Aliquots (25 μl) were rapidly cooled on ice and the residual hemagglutinating activity was assayed as previously described.

In order to determine the pH stability of AEL, buffers with a pH ranging from 2.0 to 12.0 were used as follows: 0.1 M sodium citrate buffer (pH 2.0–5.0), 0.1 M sodium monobasic phosphate buffer (pH 6.0–8.0), 0.1 M sodium carbonate/bicarbonate buffer (pH 9.0–11.0) and 0.1 M KCl/NaOH buffer (pH 12.0). A volume of 50 μl of lectin solution (100 μg) was incubated with 50 μl of buffer for 1 h at room temperature. The samples were adjusted to pH 7.0 and the residual hemagglutinating activity was measured.

The effect of two denaturing agents, urea and guanidine-HCl (Gdn.HCl), at a concentration range of 0–6 M, was tested on lectin activity by incubating 25 μl of each denaturant solution with an equal volume of AEL at 37 ºC for 2 h. The denaturant-treated samples with 6 M denaturants were recorded using a spectrophotofluorometer (Model 4500; Hitachi, Tokyo, Japan).

Modification of tryptophan (Trp) residues

Oxidation of AEL by NBS was carried out according to the method of Spande and Witkop [20]. AEL (1 mg/ml) was dissolved in sodium acetate buffer (pH 5.1, 0.1 M) and divided into four aliquots. Aliquot 1 was taken as the control. Aliquots 2 and 3 were used for modification of Trp residues in the presence and absence of mannose (0.2 M). Urea (8 M) was added into aliquot 4 to denature the protein. NBS (5 μl, 10 mM) was added into aliquots 2–4 every 10 min. After every addition an aliquot was removed and quenched with 20 μl of tryptophan (50 mM) solution, then the residual activity and fluorescence spectrum were determined after removal of excess reagents by dialysis. The number of oxidized Trp residues was calculated using the molar extinction coefficient for Trp residue (5500 per M/cm) as described by Spande and Witkop [20].

Modification of arginine (Arg) residues

Arg residues of the protein were modified by reaction with p-nitrophenylglyoxal using the method of Yamasaki *et al.* [21]. AEL (1 mg/ml) was dissolved in 0.1 M sodium pyrophosphate buffer (pH 9.0) and 25 μl of p-nitrophenylglyoxal (10%; *V*/*V*) was added every 30 min, both in the presence and absence of mannose (0.2 M). The number of modified Arg residues was determined as described by Yamasaki *et al.* [21].

Modification of carboxyl groups

Modification of carboxyl groups was carried out by the method described by Matsuo *et al.* [22]. The lectin (1 mg/ml) was incubated with or without mannose (0.2 M) in a reaction mixture containing 50 mM MES buffer (pH 6.5). Twenty-five microliters of EDC (100 mM) and 80 μM [1-^{14}C]glycine ethyl ester (sp. Radioactivity, 41.59 Ci/mmol) was added every 30 min. The amount of radioactively labeled protein was determined in Bray's solution with a Beckman LS 7000 liquid-scintillation counter (Fullerton, USA) and the number of modified residues was calculated [23].

Modification of cysteine (Cys) residues

The thiol side chains of Cys residues of AEL were modified with Ellman's reagent DTNB in the presence or absence of mannose (0.2 M) under native conditions (0.1 M PBS, pH 8.5). After denaturing and reducing the protein with 8 M urea and 10 mM β-mercaptoethanol, the free thiol groups were then modified with DTNB by incubating the lectin (1 mg/ml) with 50 mM DTNB for 1 h at room temperature [24]. After extensive dialysis of the modified samples against PBS (0.1 M, pH 7.0), the hemagglutinating activity was assayed and the number of Cys residues was calculated [25].

Chemical modification of other amino acids

Modification of threonine/serine residues was carried

out by the method of Kraut *et al*. [26]. According to the method described by Melchior *et al.* [27], histidine residues were modified by diethyl pyrocarbonate.

Fluorescence spectroscopy

Relative fluorescence intensity was measured by a Hitachi Model 4500 spectrophotofluorometer using a 5 nm slit width. Two milliliters of Trp residue-modified and denatured AEL (with 6 M urea and Gdn.HCl) was placed in a 1.0 cm×1.0 cm×4.5 cm quartz cuvette. The samples were excited at 295 nm and the emission spectra were recorded between 300 nm and 400 nm.

Circular dichroic (CD) spectroscopy

The far-ultraviolet (UV) (200–250 nm) CD spectra of the modified AEL with various reagents were measured with a Jasco-700 spectropolarimeter (Jasco, Tokyo, Japan) using 2 mm path length quartz cuvette, at a constant temperature maintained at 25 ºC and a protein concentration of 0.1 mg/ml. The CD results were expressed in millidegree as well as mean residual ellipticity. For each spectrum, five successive scans were collected and the averaged spectra were used for further analysis.

Determination of N-terminal sequence

Following SDS-PAGE, the lectin was transferred to a polyvinylidene difluoride membrane (Millipore, Bedford, USA) stained with Coomassie Brilliant Blue R-250. The band corresponding to the lectin was then excised from the membrane. The N-terminal sequence was determined by automated Edman degradation using a Hewlett-Packard 1000A protein sequencer equipped with a high performance liquid chromatography system. N-terminal sequence homology was analyzed using the BLAST database search.

In vitro antiproliferative potential of AEL on human cancer cell lines

The inhibitory potential of AEL against various human cancer cell lines such as Pro-01, Lu-04, Bre-04, HepG2 and HeLa was tested using the method of Kaur *et al.* [28]. Cells were seeded at 10^4 cells/well in 100 μl of RPMI 1640 medium containing 10% fetal calf serum in a 96-well tissue culture plate, suspended as a single cell in the medium and incubated for 24 h in a CO_2 incubator. Subsequently, 100 μl of lectin solution (1–50 μg/ml), prepared in RPMI 1640 medium, was added to cells and the cultures were incubated for 48 h. After the incubation period, adherent cell cultures were fixed *in situ* by adding 50 μl of 50% (*V*/*V*) trichloroacetic acid (final concentration 10%) and incubated for 1 h at 4 ºC. The supernatant was discarded and the plates were washed five times with deionized water and dried. One hundred microliters of sulforhodamine B (0.4% *W*/*V* in acetic acid) was added to each well and the cultures were incubated for 30 min at room temperature. The unbound sulforhodamine was removed by washing with 1% acetic acid and the plates were air-dried. The dye bound to basic amino acids of the cell membrane was solubilized with Tris buffer (10 mM, pH 10.5) and the absorption was measured at 540 nm using an enzyme-linked immunosorbent assay reader to determine the relative cell growth viability in the treated and untreated cells.

Results

Purification of agglutinin from *A. elatior* rhizomes

The 80% ammonium sulfate saturated crude protein extract after dialysis against 20 mM Tris-HCl (pH 8.0), was applied to a diethylaminoethyl-Sepharose column that was previously equilibrated with the same buffer. Three adsorbed peaks were eluted with a linear 0–0.5 M NaCl gradient from the column, but only D3 was detected with hemagglutinating activity. No activity was found in the much larger peak D2 [**Fig. 1(A)**]. D3 was then loaded on the carboxymethyl-Sepharose column and four peaks were eluted with the equilibrating buffer containing 100 mM, 250 mM and 400 mM NaCl [**Fig. 1(B)**]. C4 showed strong agglutinating activity, however, low hemagglutinating activity was also detected in C5. The mixture of C4 and C5 was resolved into two peaks, S1 and S2, on gel filtration Sephacryl S-100. S2, in which lectin activity resided, was higher than S1 [**Fig. 1(C)**].

Homogeneity and molecular weight determination

Both in the presence and absence of β-mercaptoethanol, AEL yielded two adjacent bands with molecular masses of 13.5 kDa and 14.5 kDa (**Fig. 2**) in SDS-PAGE, suggesting that the subunits are not linked by a disulphide bond. The apparent molecular mass of AEL, as determined by gel filtration chromatography on a calibrated Sephacryl S-100 column, was 56 kDa (**Fig. 3**).

Hemagglutination and hemagglutination-inhibition assays

The lectin AEL agglutinated specifically the rabbit erythrocytes, with a minimum concentration of 3.9 μg/ml needed for visible agglutination. Carbohydrate-binding

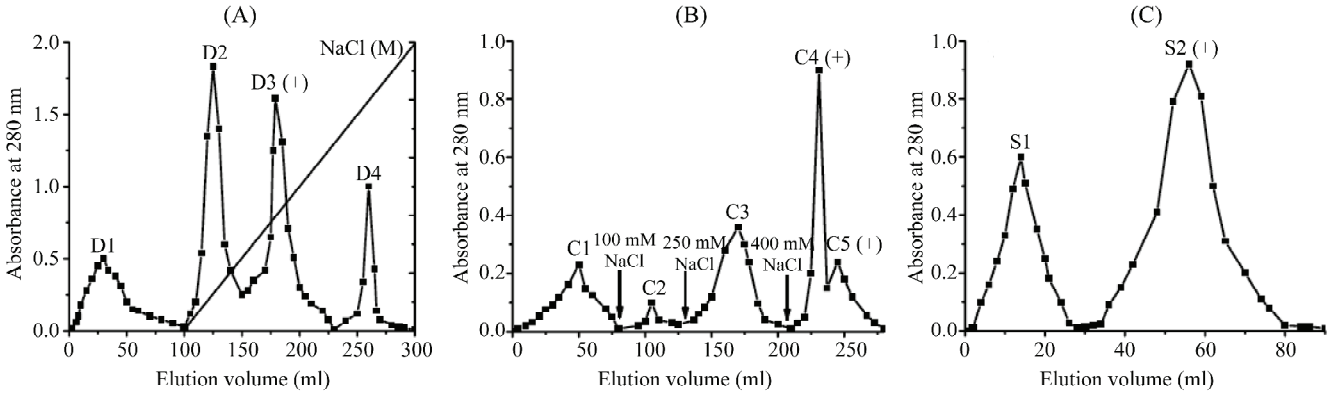

Fig. 1 Ion exchange and gel filtration chromatography of *Aspidistra elatior* Blume lectin

The elution profiles were monitored at 280 nm. The active fractions were detected by hemagglutination assay. (A) Anion exchange chromatography of the crude extract (100 ml, 1.2 mg/ml) of *A. elatior* rhizomes on the diethylaminoethyl (DEAE)–Sepharose column (2 cm×20 cm). The unadsorbed fraction was eluted with 20 mM Tris-HCl buffer (pH 8.0), and the adsorbed fraction was eluted with 0–0.5 M NaCl gradient at a flow rate of 2 ml/min. (B) Cation exchange chromatography of the active fraction D3 (50 ml, 0.3 mg/ml) from the DEAE–Sepharose column on the carboxymethyl (CM)–Sepharose column (2 cm×20 cm). C1 was eluted with 20 mM sodium acetate buffer (pH 4.6). C2, C3, C4 and C5 were eluted with 100, 250 and 400 mM NaCl in sodium acetate buffer. (C) Gel filtration chromatography of the mixture C4 and C5 (2 ml, 4 mg/ml) from the CM–Sepharose column on a Sephacryl S-100 column (2 cm×100 cm). Phosphate-buffered saline (10 mM, pH 7.2) was used as running buffer at a flow rate of 0.5 ml/min.

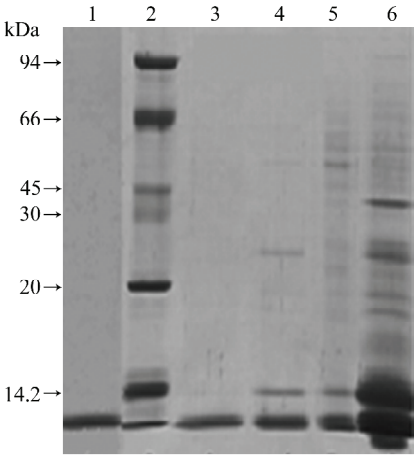

Fig. 2 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the various purification steps of *Aspidistra elatior* Blume lectin (AEL)

The amount of purified lectin loaded per lane was 50 μg. The gels were stained with Coomassie brilliant blue R-250 (2.5 mg/ml). 1, gel filtration purified AEL (without β-mercaptoethanol); 2, low molecular weight markers; 3, gel filtration purified AEL (S2) in the presence of β-mercaptoethanol; 4, active fractions (C4, C5) from the carboxymethyl–Sepharose column; 5, an active fraction (D3) from the diethylaminoethyl–Sepharose column; 6, ammonium sulfate precipitation (80%).

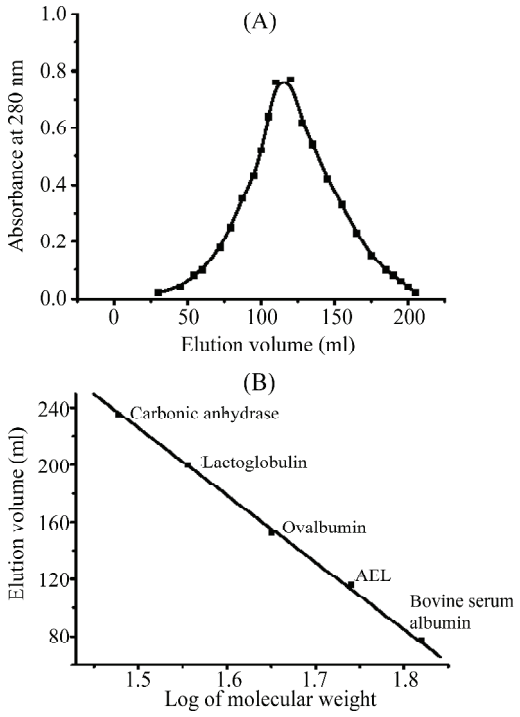

Fig. 3 Elution profile and molecular mass estimation of *Aspidistra elatior* Blume lectin (AEL) using a Sephacryl S-100 column

(A) The elution profile of the purified AEL on a Sephacryl S-100 column. The elution profile was monitored at 280 nm and phosphate-buffered saline (10 mM, pH 7.2) was used as running buffer at a flow rate of 0.2 ml/min. (B) Native molecular mass estimation of AEL by standard plot on the gel filtration Sephacryl S-100 column. Bovine serum albumin (66 kDa), ovalbumin (45 kDa), lactoglobulin (36 kDa) and carbonic anhydrase (30 kDa) were used as standard proteins.

specificity assay with a series of sugars (arabinose, maltose, mannose, N-acetyl-*D*-glucosamine, 2-methyl-*D*-glucoside, N-acetygalactosamine, N-acetylactosamine, *D*-galactose, *D*-galactosamine, lactose, *D*-fructose, sucrose, mannan) revealed that only mannose and mannan had

inhibitory effects in all the monosaccharides, disaccharides and oligosaccharides tested. The minimum sugar concentrations for complete inhibition of the activity (4 hemagglutination units) were 50 mM and 100 μg/ml (**Table 1**).

Table 1 Sugar specificity of Aspidistra elatior Blume lectin (AEL) and minimal inhibitory sugar concentration (MISC) required for complete hemagglutination inhibition

S. No	Sugar/glycoprotein	MISC
1	Mannose	50 mM
2	Mannan	100 μg/ml
3	Ovomucoid	250 μg/ml
4	Thyroglobulin	12.5 μg/ml

The following sugars or glycoprotein were not inhibitory at a final concentration of 200 mM or 200 μg/ml: arabinose; maltose; N-acetyl-*D*-glucosamine; 2-methyl-*D*-glucoside; *N*-acetygalactosamine; *N*-acetylactosamine; *D*-galactose; *D*-galactosamine; *D*-fructose; lactose; sucrose; fetuin; and sialofetuin.

Similar assays were also carried out with some glycoproteins. Thyroglobulin was the most potent inhibitor, being active at a concentration of 12.5 μg/ml, and ovomucoid showed a weak inhibitory effect (**Table 1**). Other sugars and glycoproteins tested were devoid of any inhibitory effect.

Effect of temperature, pH and denaturants on hemagglutinating activity of AEL

The results of thermal denaturation of AEL showed that the hemagglutinating activity was extremely stable between 10 ºC and 70 ºC. Even heating at 80 ºC for 30 min caused a loss of only 25% of its original activity. However, its hemagglutinating activity was completely lost when it was exposed to 90 ºC [**Fig. 4(A)**].

The examination of AEL activity toward different pH values (pH 2.0–12.0, showed that the lectin exhibited maximum activity in a narrow pH range of 7.0–8.0, as there was a decline in lectin activity below and above this pH range [**Fig. 4(B)**].

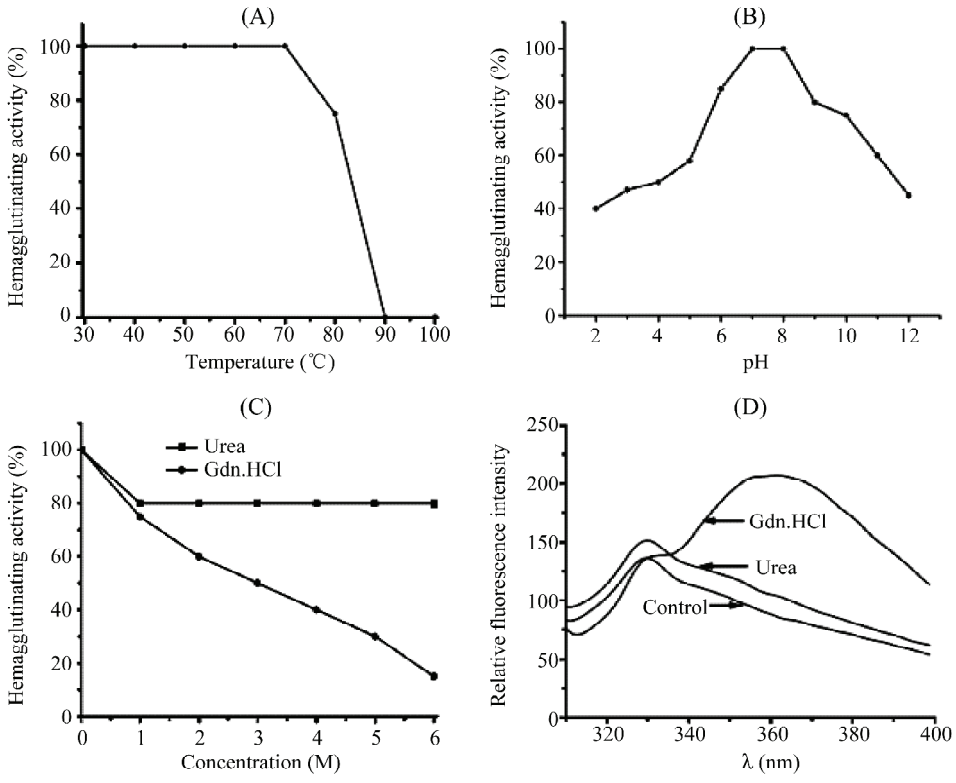

Fig. 4 Effects of temperature, pH and denaturants on the hemagglutinating activity of *Aspidistra elatior* Blume lectin

(A) Thermal stability of *Aspidistra elatior* Blume lectin (AEL). The lectin was incubated at an elevated temperature (30–100 ºC) for 30 min, cooled and its hemagglutinating activity was tested. The line represents the percentage hemagglutination activity of AEL. (B) Effect of pH on the hemagglutinating activity of AEL. Buffers ranging from pH 2.0 to 12.0 were used (see "Materials and Methods"). The line represents the percentage hemagglutination activity of the lectin. (C) Effect of two denaturants (urea and Gdn.HCl) on the hemagglutinating activity of AEL. Concentration of denaturants used was 0–6 M. The line represents the percentage hemagglutination activity of the lectin. (D) Relative fluorescence spectra of AEL in the presence of Gdn.HCl and urea (6 M). Extraction was carried out at 295 nm and the emission spectra were recorded between 300 nm and 400 nm.

The hemagglutinating activity of AEL had a slight decrease after treatment with 6 M urea, and only 20% of the hemagglutinating activity was lost. However, the activity dropped nearly 85% after incubation with 6 M Gdn.HCl [**Fig. 4(C)**]. **Fig. 4(D)** shows the fluorescence spectra of AEL when treated with urea and Gdn.HCl at the final concentration of 6 M. It was clearly evident that the fluorescence intensity nearly doubled and the λ_{max} experienced a large red shift from 330 nm to 362 nm when the lectin was incubated with 6 M Gdn.HCl. However, the urea-treated sample remained almost unchanged compared with the control.

Modification of Trp residues

After treatment of AEL with NBS, a reagent specifically modifying Trp residue, and removal of excess reagent, a total loss of agglutinating activity was observed. According to the method of Spande and Witkop [20], the number of Trp residues modified by NBS could be calculated through **Equation 1**:

$$n=(1.31\times\Delta A_{280}\times Mr)/(C\times 5500) \quad 1$$

where n was the number of Trp residues modified by NBS, ΔA_{280} was the change of absorbance at 280 nm, Mr was the relative molecular weight, and C was the concentration of the protein. A plot of the number of Trp residues modified by NBS versus remaining percentage residual activity of AEL indicated that approximately four Trp residues were modified in native conditions, approximately 33% and 100% hemagglutinating activity was lost after modification of the third and fourth Trp residue, respectively [**Fig. 5(A)**]. The fluorescence emission had a marked decrease as the Trp residues/molecules were sequentially modified [**Fig. 5(B)**]. The number of modified Trp residues

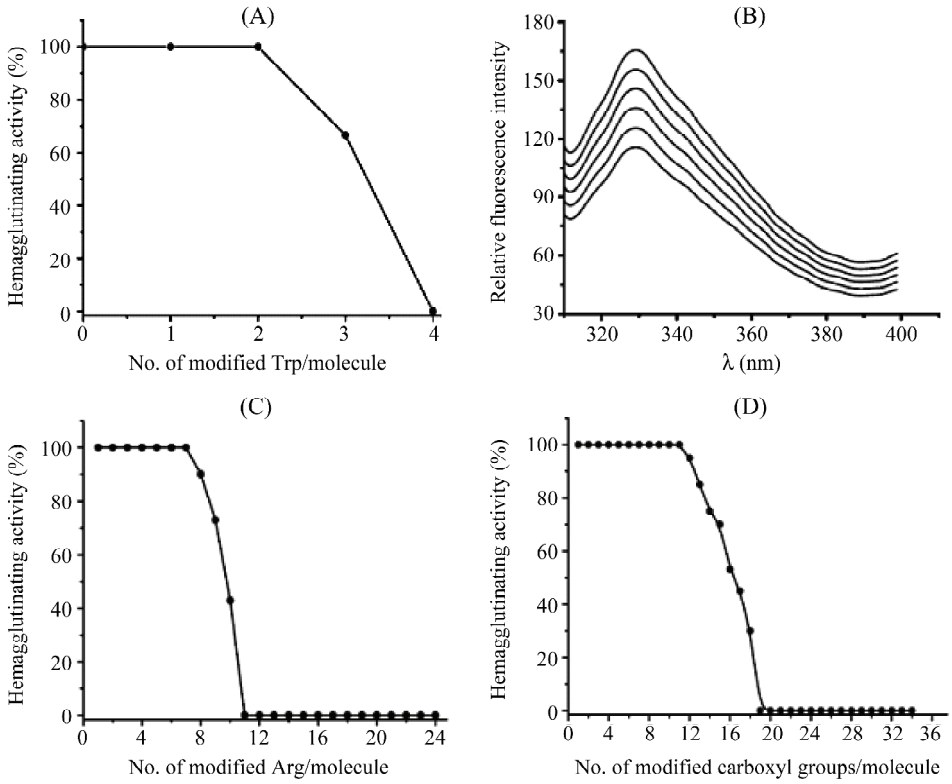

Fig. 5 Effect of modification of Trp and Arg residues and carboxyl groups on hemagglutinating activity of *Aspidistra elatior* Blume lectin (AEL)

(A) Effect of modification of Trp residues on the hemagglutinating activity of AEL under native conditions (sodium acetate buffer, 0.1 M, pH 5.1, without mannose and urea). The line represents the percentage residual hemagglutinating activity of AEL. (B) Fluorescence spectra of AEL with different concentrations of NBS (without mannose and urea). Curves from top to bottom represent the fluorescence spectra after the addition of 0, 5, 10, 15, 20 and 25 μl of 10 mM NBS. Excitation was carried out at 295 nm and the emission spectra were recorded between 300 and 400 nm. (C) Effect of modification of Arg residues on the hemagglutinating activity of AEL under native conditions (sodium pyrophosphate buffer, 0.1 M, pH 9.0, without mannose and urea). The line represents the percentage residual hemagglutinating activity of AEL. (D) Effect of modification of carboxyl groups on the hemagglutinating activity of AEL under native conditions (MES buffer, 50 mM, pH 6.5, without mannose and urea). The line represents the percentage residual hemagglutinating activity of AEL.

in the presence of mannose did not change, which suggested that Trp residues might not be present in the active sites of AEL. To estimate the total number of modified Trp residues, urea (8 M) was used to denature the protein. Approximately 12 Trp residues were modified under denaturing conditions (**Table 2**).

Modification of Arg residues

Modification of AEL with p-nitrophenylglyoxal under native conditions (sodium pyrophosphate buffer, 0.1 M, pH 9.0) resulted in the modification of approximately 23.5 and 23.8 Arg residues/molecule in the presence and absence of mannose, respectively. The number of residues modified after denaturing the protein was almost the same. Modifications with and without mannose under native conditions both led to complete loss of the hemagglutinating activity of the lectin (**Table 2**). When the hemagglutination activity was monitored as a parameter to evaluate the extent of modification, it was observed that the modification of seven Arg residues/molecules did not alter the activity of the lectin, but thereafter the activity decreased with further modification. Modification of eight Arg residues resulted in a 10% decrease in the agglutination activity. Modification of nine and 10 Arg residues decreased the hemagglutination activity of the lectin by 27% and 57%, respectively. Modification of 11 Arg residues led to the complete abrogation of the hemagglutination activity of AEL [**Fig. 5(C)**].

Modification of carboxyl groups

Modification of the carboxyl groups under native conditions (MES buffer, 50 mM, pH 6.5) showed that approximately 42 carboxyl groups/molecules were modified and complete loss of the hemagglutinating activity was observed. However, only 34 carboxyl groups/molecules were modified and only 50% hemagglutinating activity was lost in the presence of mannose under the same conditions (**Table 2**). The sequential modification of carboxyl group results suggested that modification of 11 carboxyl groups/molecules did not alter the activity of the lectin, but modification of 12–19 residues/molecules resulted in decreased hemagglutinating activity by 5%, 15%, 25%, 30%, 47%, 55% and 70%, respectively. Complete loss of the lectin activity was observed after modification of 20 carboxyl groups/molecules [**Fig. 5(D)**].

Modification of Cys residues

Treatment of the lectin with DTNB resulted in the modification of 4.2 and 4.1 Cys residues/ molecules in the presence and absence of mannose, respectively. A 60%

Table 2 Summary of results obtained from the chemical modification studies on *Aspidistra elatior* Blume lectin (AEL)

Reagent	Residues modified	No. of residues modified/molecule	Remaining activity (%)
NBS			
Native	Trp	4.1	0
(+)*D*-mannose	Trp	4.3	0
(+)urea	Trp	11.9	N.D.
p-Nitrophenylglyoxal			
Native	Arg	23.8	0
(+)*D*-mannose	Arg	23.5	0
(+)urea	Arg	24.1	N.D.
EDC			
Native	Asp/Glu	41.6	0
(+)*D*-mannose	Asp/Glu	33.7	50
DTNB			
Native	Cys	4.1	40
(+)*D*-mannose	Cys	4.2	40
(+)urea, (–)β-mercaptoethanol	Cys	9.8	N.D.
(+)urea, (+)β-mercaptoethanol	Cys	9.9	N.D.

Modification of other amino acids had no effect on the hemagglutinating activity of AEL and are not included in this table. Concentrations of *D*-mannose, urea and β-mercaptoethanol used in this study were 0.2 M, 8 M and 10 mM, respectively. +, with; –, without; DTNB, 5,5'-dithiobis(2-nitrobenzoic acid); EDC, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide; NBS, *N*-bromosuccinimide; N.D., not determined.

drop in AEL activity was clear upon modification of Cys residues in both conditions which suggested partial necessity of Cys residue(s) in the activity of AEL. After denaturing the protein with 8 M urea, approximately 9.9 Cys residues/molecules were modified (**Table 2**). Addition of β-mercaptoethanol (10 mM) had no effect on the number of modified Cys residues.

Chemical modification of other amino acids

Modification of threonine/serine and histidine residues did not affect the hemagglutinating activity of the lectin. These results indicated that these residues were not involved in the hemagglutinating activity of AEL (data not shown).

CD spectroscopy

Changes in the CD spectra when AEL was modified by various reagents are shown in **Fig. 6**. The far-UV CD spectra of native AEL showed the broad negative minimum at 215 nm. After modification of Trp residues, the CD spectrum underwent a significant change: two negative minima at 207 nm and 222 nm with moderate increase in ellipticity (θ) were observed. Modification of Arg residues also led to two negative minima at 209 nm and 216 nm with slight increase in observed ellipticity (θ), and Cys-modified AEL at far-UV resulted in two broad minima at 206 nm and 218 nm with further increase of the observed ellipticity (θ). However, the CD spectrum of EDC-modified lectin in the far-UV region was nearly identical to the spectrum of the native AEL (**Fig. 6**).

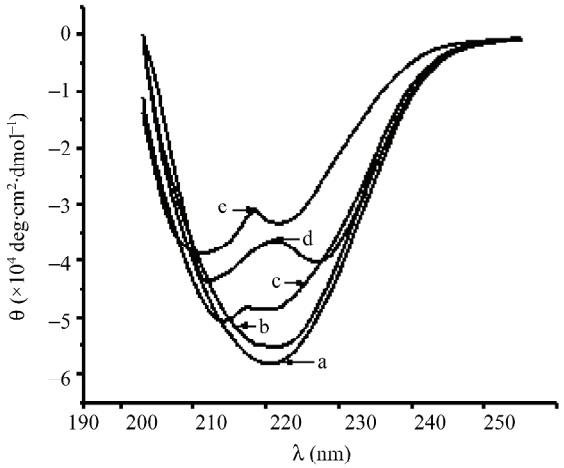

Fig. 6 Effect of chemical modification on the far-ultraviolet (200-250 nm) circular dichroic (CD) spectra of *Aspidistra elatior* Blume lectin (AEL)

All the CD spectra were measured at a constant temperature maintained at 25 ºC and a protein concentration of 0.1 mg/ml under native conditions (without mannose and urea). Curve a, native protein; curve b, 42 carboxyl groups/molecule modified AEL; curve c, 23.8 Arg residues/molecule modified AEL; curve d, 4 Trp residues/molecule modified AEL; curve e, 4.2 Cys residues/molecule modified AEL.

N-terminal sequence determination

The N-terminal sequences of the two subunits of AEL are presented in **Table 3**. AEL exhibited only a low degree

Table 3 Comparison of N-terminal amino acid sequence of *Aspidistra elatior* lectin (AEL) 13.5 kDa and 14.5 kDa subunits with other related monocot lectins

Family	Lectin	Sequence	Identity (%)
Liliaceae	AEL-13.5 kDa	YNFLSSPKSLRIPQALTTG	100
	AEL-14.5 kDa	DNILTSPNSLLSDQALTTD	58
	PML	DNSLTSPNSLPSGHSLNTG	47
	PCL	VNSLSSPNSLFTGHSLEVG	47
	TxLMII(2B)	PNNVLYTGESLYGGQSLT	35
	TxLCI(B)	QNVLLSGNTLANEESLSYG	32
	SGA-15 kDa	NNVLETQESLQSDERLSYQ	26
	SGA-17 kDa	NQVLLTQESLQSDERLSYQ	21
Amaryllidaceae	GNA	DNILYSGETLSTGEFLNYG	32
Orchidaceae	LOA(L)	LNHLLGGERLNTGQSLTDG	37
Alliaceae	ASA	RNVLTTGETLHAGEHLDIG	26

Residues identical to those of corresponding residues in AEL 13.5 kDa (i.e., 13.5 kDa subunit of AEL) are underlined. Data of TxLMII(2B) (the second Tulipa hybrid lectin) and TxLCI(B) (the first Tulipa hybrid lectin), *Polygonatum multiflorum* lectin (PML), *Polygonatum cyrtonema* lectin (PCL) and *Smilax glabra* agglutinin (SGA) are from references [29,12,13,9], respectively, and those of GNA, LOA(L) and ASA are from references [30,31,32] respectively.

Table 4 ***In vitro*** **antiproliferative potential of** ***Aspidistra elatior*** **lectin against human cancer cell lines Pro-01, Lu-04, Bre-04, HepG2 and HeLa**

Lectin concentration (μg/ml)	Growth inhibition against cell lines (%)				
Pro-01	Lu-04	Bre-04	HepG2	HeLa	
Control†	0	0	0	0	0
1	0	4	16	15	0
10	0	42	40	20	3
25	20	53	58	50	8
50	33	60	66	56	10

† In the control wells, cells were cultured with medium alone (no lectins). The growth inhibition of the cells was measured by an enzyme-linked immunosorbent assay reader.

of resemblance to some Liliaceae lectins and lectins from Amaryllidaceae, Orchidaceae and Alliaceae.

In vitro antiproliferative potential of AEL on human cancer cell lines

The antiproliferative effect of the lectin on human cancer cells was determined over a range of 1–50 μg/ml. AEL showed maximum antiproliferative effect against Bre-04 (66%), followed by Lu-04, HepG2, and Pro-01, in which 60%, 56% and 33% growth inhibition was observed, respectively, at the highest concentration of 50 μg/ml. At a concentration of 25 μg/ml, the growth inhibition observed was 58% for Bre-04, 53% for Lu-04, 50% for HepG2 and 20% for Pro-01. However, at a lower concentration of 10 μg/ml, the growth inhibition was 42% and 40% in Lu-04 and Bre-04 cell lines, respectively. The lectin was found to be inactive against HeLa cell line at all the concentrations studied (**Table 4**).

Discussion

A novel lectin with antiproliferative activity was purified from the rhizomes of a traditional Chinese medicinal herb, *A. elatior* Blume, a member of family Liliaceae. In recent years, much of the research into the biochemical constituents of *A. elatior* Blume focused on steroidal compounds [14] and there was no report on the bioactive proteins. This lectin might represent the first isolated proteinaceous constituent of *A. elatior* Blume. The isolation procedure for this lectin consisted of three chromatographic steps. In each step, some proteins devoid of hemagglutinating activity were eliminated, indicating that the purification procedure was effective. Molecular weight determination showed that AEL was a heterotetramer and possessed two slightly different subunits (13.5 kDa and 14.5 kDa) that were not linked by disulphide bond. Apparently, according to its subunit and molecular structure, this lectin is similar to some rhizome lectins, such as *Acorus calamus* lectin, *Acorus gramineus* lectin [33] and *Par. quadrifolia* lectin [11] isolated from *Aco. calamus* (Linn.), *Aco. gramineus* (Solandin Ait.) (Araceae) and *Par. quadrifolia* L (Liliaceae), respectively. According to previous reports, most of the characterized monocot mannose-binding lectins such as *Colocasia esculenta* agglutinin and *Arum maculatum* agglutinin were heterotetramers composed of four polypeptides chains of which two chains were identical but different from the other two chains. Each chain had a similar size of 11–14 kDa [34]. As with many other monocot mannose-binding lectins, the negative result of the determination of the total neutral sugar content (data not shown) indicates that this lectin is also unglycosylated.

The hemagglutination inhibition assays showed that the lectin activity was inhibited by mannose and mannose polymers (mannan). Like many other monot mannose-binding lectins, the inhibition effect of mannan was much stronger than mannose, which indicated that the carbohydrate-binding sites of AEL accommodate preferentially oligomannosyl residues [6]. Among the glycoproteins tested, thyroglobulin was the most potent inhibitor and ovomucoid showed weak inhibition. Other glycoproteins showed no inhibition even at a concentration of 4 mg/ml. The carbohydrate specificity of AEL was similar to those of *Allium ascalonicum* agglutinin [35], the second mannose-binding Tulipa hybrid lectin [8], PML [12] and the second *S. glabra* agglutinin [10] which were also mannose-binding lectins from Liliaceae. But some other lectins from Liliaceae, such as PCL [13] and *Polygonatum verticillatum* lectin [36], can interact with sialic acid. In addition, the first reported rhizomatic lectin, isolated from *S. glabra*, found no inhibitory sugars or glycoproteins [9].

The results of thermal denaturation of AEL suggested that the lectin is significantly stable up to 70 ºC. Only 25% hemagglutinating activity was lost when heated at 80 ºC for 30 min. By comparison, SGA lost 75% hemagglutinating activity at 70 ºC and the activity was totally lost at 80 ºC or above [9]. The lectin showed optimum activity in a narrow pH range of 7.0–8.0. This is similar to the rhizomatic lectin isolated from *Arundo donax*, whose optimum pH range for activity is pH 7.0–9.0 [37]. Moreover, this lectin resembles some mannose-binding lectins with respect to its pH stability from the Amaryllidaceae and Alliaceae families [38]. Denaturation of AEL by two denaturants (urea and Gdn.HCl) showed that Gdn.HCl was much more powerful in denaturing the protein. The stronger denaturing ability of Gdn.HCl indicates that charge interactions are also important, as well as hydrophobic forces, in stabilizing the native structure of the lectin. These denaturants are known to disturb the 3-D conformation and binding sites of lectins by affecting the hydrophobic interactions that play crucial roles in carbohydrate binding activity.

Modifications of crucial amino acids with group-specific reagents might bring about changes in the biological character of lectins, which can be monitored by analyzing the hemagglutinating activity and spectrum alterations of lectins. Thus, chemical modification provides a useful approach for the identification of amino acid residues present in or near the active site of lectins [39].

Treating purified AEL with NBS, a reagent specific for Trp residues in restricted conditions, completely inactivated the lectin with total modification of four Trp residues in native conditions. Approximately 12 Trp residues were modified after denaturing the protein with 8 M urea. Of these residues the third or fourth modified residue seemed to play a crucial role in the hemagglutinating activity of AEL. The presence of the haptenic sugar mannose in the assay medium did not provide protection for AEL against NBS, which indicated that Trp residues might not be present in the mannose-binding site of the lectin. The loss in hemagglutinating activities of AEL might be due to the secondary structure change, as the CD spectrum of the protein in the far-UV region (200–250 nm) underwent a significant change. It is rational to conclude that these Trp residues were not in the carbohydrate-binding sites and not directly involved in carbohydrate recognition and binding. They were essential for the hemagglutinating activity of AEL by being located near the carbohydrate-binding sites of the lectin and involved directly in maintaining the crucial conformation of the carbohydrate-binding center instead of binding the mannose directly.

Modification of Arg and Cys residues suggested that some of these residues might also be present near the carbohydrate-binding sites and not directly involved in the saccharide recognition and binding. The loss of the hemagglutinating activity caused by modification could be due to the secondary structural changes, as the CD spectra of the modified samples were obviously changed compared with control (**Fig. 6**). These residues might also play an important role, like Trp residues, in maintaining the active conformation of the protein to exhibit hemagglutinating activity. This conclusion was further supported by the fact that Arg and Cys residues were not involved in the carbohydrate binding site of many other mannose-binding lectins [6]. Modifications of Cys residues, carried out under denaturing and reducing conditions, indicated that there were no disulfide bonds present in the molecule. These results give further support to the conclusion of electrophoresis discussed above.

Modification of carboxyl groups also caused 100% reduction in hemagglutinating activity. The complete loss of the hemagglutinating activity might have resulted from the modification rather than the conformation changes, because the CD spectrum was nearly identical to the spectrum of the native AEL (**Fig. 6**). Modifications carried out in the presence of mannose indicated that there were eight carboxyl groups/molecules located in the carbohydrate-binding site. This carbohydrate protection effect was consistent with the fact that some carboxyl groups are located in the mannose-binding site of many mannose-binding lectins, such as *Galanthus nivalis* agglutinin [30], PCL [13] and PML [12].

AEL is a novel lectin with a unique N-terminal sequence, which showed only a low degree of resemblance to some Liliaceae lectins, including PCL (47%), PML (47%), the second mannose-binding Tulipa hybrid lectin (35%), the first Tulipa hybrid lectin (32%) and SGA (26% with SGA-15 kDa, 21% with SGA-17 kDa) (**Table 3**). In addition, AEL shared only approximately 30% identity to some other lectins from Amaryllidaceae, Orchidaceae and Alliaceae. However, lectins from the Amaryllidaceae family resemble each other much more closely (85%–90% identity in sequence) [9].

In vitro antiproliferative activity of AEL was evaluated against five human cancer cell lines representing different organs and tissues. Bre-04, Lu-04, HepG2 and Pro-01 were significantly inhibited by AEL. However, it showed no inhibitory effect towards the HeLa cell line at any of the concentrations studied. All the results showed that AEL has *in vitro* cell line specific antiproliferative potential against human cancer cell lines in a dose-dependent manner (**Table 4**). This finding is consistent with the earlier reported

variation in the antiproliferative potential of a variety of lectins with cancer cell lines [40]. At present, it is difficult to explain why AEL showed significant inhibitory effect on the proliferation of Bre-04, Lu-04 and HepG2 but failed to restrict the proliferation of the HeLa cell line. Differences in terminal sugars in various tumor cell lines, as reported in published work, could be one of the reasons for the antiproliferative activity of AEL [41]. This lectin is similar to the garlic lectin which also showed *in vitro* cell line specific antiproliferative potential against human cancer cell lines in a dose-dependent manner [42].

In conclusion, AEL is a novel lectin with a low degree of similarity to other mannose-binding lectins from Liliaceae, Amaryllidaceae, Orchidaceae and Alliaceae. It manifests potent antiproliferative activity to human cancer cell lines and represents the first isolated proteinaceous constituent of *A. elatior* Blume. Further investigations are necessary to unravel the molecular basis for the antiproliferative potential of the lectin on cell differentiation and proliferation.

References

1 Dixon HBF. Defining a lectin. Nature 1981, 292: 192

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

3 Abdullaev FI, Mejia EG. Antitumor effect of plant lectins. Nat Toxins 1997, 5: 157–163

4 Schumacher U, Higgs D, Loizidou M, Pickering R, Leathem A, Taylor I. *Helix pomatia* agglutinin binding is a useful prognostic indicator in colorectal carcinoma. Cancer 1994, 74: 3104–3107

5 Parslew R, Jones KT, Rhodes JM, Sharpe GR. The antiproliferative effect of lectin from the edible mushroom (*Agaricus bisporus*) on human keratinocytes: Preliminary studies on its use in psoriasis. Br J Dermatol 1999, 140: 56–60

6 Van Damme EJ, Peumans WJ, Barre A, Rouge P. Plant lectins: A composite of several distinct families of structurally and evolutionary related proteins with diverse biological roles. Crit Rev Plant Sci 1998, 17: 575–692

7 Koike T, Beppu H, Kuzuya H, Maruta K, Shimpo K, Suzuki M. A 35 kDa mannose-binding lectin with hemagglutinating and mitogenic activities from "Kidachi Aloe" (*Aloe arborescens* Miller var. natalensis Berger). J Biochem 1995, 118: 1205–1210

8 Oda Y, Minami K. Isolation and characterization of a lectin from tulip bulbs *Tulipa gesneriana*. Eur J Biochem 1986, 159: 239–245

9 Ng TB, Yu YL. Isolation of a novel heterodimeric agglutinin from rhizomes of *Smilax glabra*, the Chinese medicinal material tufuling. Int J Biochem Cell Biol 2001, 33: 269–277

10 Ooi LS, Sun SS, Wang H, Ooi VE. New mannose-binding lectin isolated from the rhizome of sarsaparilla *Smilax glabra* Roxb. (Liliaceae). J Agric Food Chem 2004, 52: 6091–6095

11 Antoniuk V. Purification of lectin from *Paris quadrifolia* L. and comparison of its carbohydrate-binding specificity with other lectins of the Liliaceae family. Ukr Biokhim Zh 1996, 68: 86–91

12 Van Damme EJ, Barre A, Rougé P, Van Leuven F, Balzarini J, Peumans WJ. Molecular cloning of the lectin and a lectin-related protein from common Solomon's seal (*Polygonatum multiflorum*). Plant Mol Biol 1996, 31: 657–672

13 An J, Liu JZ, Wu CF, Li J, Dai L, Van Damme E, Balzarini J *et al*. Anti-HIV I/II activity and molecular cloning of a novel mannose/sialic acid binding lectin from rhizome of *Polygonatum cyrtonema* Hua. Acta Biochim Biophys Sin 2006, 38: 70–78

14 Chen MQ, Sichuanensis KY, Lang Y. Steroidal glycosides from *Aspidistra*. Nat Prod Res Dev 1995, 7: 19–22

15 Laemmli UK, Favre M. A method of protein separation. J Mol Biol 1973, 80: 575–599

16 Whitakar JR. Determination of molecular weights of proteins by gel filtration on Sephadex. Anal Chem 1963, 35: 1950–1953

17 Andrews P. Estimation of molecular weights of proteins by Sephadex gel-filtration. Biochem J 1964, 91: 222–233

18 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with folin-phenol reagent. J Biol Chem 1951, 193: 265–275

19 Dubois M, Giles K, Hamilton JK, Robers PA, Smith F. Colorimetric method for determination of sugar and related substances. Anal Chem 1956, 28: 350–356

20 Spande TF, Witkop B. Determination of the tryptophan content of proteins with *N*-bromosuccinimide. Methods Enzymol 1967, 11: 498–532

21 Yamasaki RB, Shimer DA, Feeney RE. Colorimetric determination of arginine residues in proteins by p-nitrophenylglyoxal. Anal Biochem 1981, 111: 220–225

22 Matsuo M, Huang C, Huang LC. Modification and identification of glutamate residues at the arginine-recognition site in the catalytic subunit of adenosine 3′:5′-cyclic monophosphate-dependent protein kinase of rabbit skeletal muscle. Biochem J 1980, 187: 371–379

23 Bray HG, James SP. The formation of mercapturic acids. Deacetylation of mercapturic acids by the rabbit, rat and guinea pig. Biochem J 1960, 74: 394–397

24 Glazer AN, DeLange RJ, Sigman DS. Techniques in biochemistry and molecular biology. North-Holland: Oxford Press, 1976

25 Sultan NA, Kenoth R, Swamy MJ. Purification, physicochemical characterization, saccharide specificity, and chemical modification of a Gal/GalNAc specific lectin from the seeds of *Trichosanthes dioica*. Arch Biochem Biophys 2004, 432: 212–221

26 Kraut D, Goff H, Pai RK, Hosea NA, Silman I, Sussman JL, Taylor P *et al*. Inactivation studies of acetylcholinesterase with phenylmethylsulfonyl fluoride. Mol Pharmacol 2000, 57: 1243–1248

27 Melchior WB, Fahrney D. Ethoxyformylation of proteins. Reaction of ethoxyformic anhydride with α-chymotrypsin, pepsin, and pancreatic ribonuclease at pH 4. Biochem 1970, 9: 251–258

28 Kaur M, Singh K, Rup PJ, Saxena AK, Khan RH, Ashraf MT, Kamboj SS. A tuber lectin from *Arisaema helleborifolium* Schott with anti-insect activity against melon fruit fly *Bactrocera cucurbitae* (Coquillett) and anti-cancer effect on human cancer cell lines. Arch Biochem Biophys 2006, 445: 156–165

29 Van Damme EJ, Birke F, Winter HC, Van Leuven F, Goldstein IJ, Peumans WJ. Molecular cloning of two different mannose-binding lectins from tulip bulbs. Eur J Biochem 1996, 236: 419–427

30 Van Damme EJ, Kaku H, Perini F, Goldstein IJ, Peeters B. Biosynthesis, primary structure and molecular cloning of snowdrop (*Galanthus nivalis* L.) lectin. Eur J Biochem 1991, 202: 23–30

31 Van Damme JM, Smeets K, Torrekens S, Van Leuven F, Peumans WJ. Characterization and molecular cloning of the mannose binding lectins from three Orchidaceae species: *Listera ovata*, *Epipactis helleborine* and *Cymbidium hybrid*. Eur J Biochem 1994, 221: 769–777

32 Smeets K, Van Damme EJ, Verhaert P, Barre A, Rouge P, Van Leuven F, Peumans WJ. Isolation, characterization and molecular cloning of the mannose-binding lectins from leaves and roots of garlic (*Allium sativum* L.).

Plant Mol Biol 1997, 33: 223–234

33 Bains JS, Dhuna V, Singh J, Kamboj SS, Nijjar KK, Agrewala JN. Novel lectins from rhizomes of two *Acorus* species with mitogenic activity and inhibitory potential towards murine cancer cell lines. Int Immunopharmacol 2005, 5: 1470–1478

34 Shangary S, Singh J, Kamboj S, Kamboj KK, Sandhu RS. Purification and properties of four monocot lectins from the family Araceae. Phytochemistry 1995, 40: 449–455

35 Mo H, Van Damme EJ, Peumans WJ, Goldstein IJ. Purification and characterization of a mannose-specific lectin from shallot (*Allium ascalonicum*) bulbs. Arch Biochem Biophys 1993, 306: 431–438

36 Antoniuk V. Purification and properties of lectins of *Polygonatum multiflorum* (L.) All and *Polygonatum verticillatum* (L.) All. Ukr Biokhim Zh 1993, 65: 41–48

37 Kaur A, Singh J, Kamboj SS, Sexana AK, Pandita RM, Shamnugavel M. Isolation of an N-acetyl-*D*-glucosamine specific lectin from the rhizomes of *Arundo donax* with antiproliferative activity. Phytochemistry 2005, 66: 1933–1940

38 Van Damme EJ, Allen AK, Peumans WJ. Isolation and characterization of a lectin with exclusive specificity towards mannose from snowdrop (*Galanthus nivalis*) bulbs. FEBS Lett 1987, 215: 140–144

39 Matthews KS, Chakerian AE, Gardner JA. Protein chemical modification as probe of structure-function relationships. Methods Enzymol 1991, 208: 468–496

40 Wang H, Ng TB, Ooi VE, Liu WK. Effects of lectins with different carbohydrate binding specificities on hepatoma, choriocarcinoma, melanoma and osteosarcoma cell lines. Int J Biochem Cell Biol 2000, 32: 365–372

41 Ngai PH, Ng TB. A mushroom (*Ganoderma capense*) lectin with spectacular thermostability, potent mitogenic activity on splenocytes, and antiproliferative activity toward tumor cells. Biochem Biophys Res Commun 2004, 314: 988–993

42 Karasaki Y, Tsukamoto S, Mizusaki K, Sugiura T. A garlic lectin exerted an antitumor activity and induced apoptosis in human tumor cells. Food Res Int 2001, 34: 7–13

Edited by
Minghua XU