

## A novel tetrameric lectin from *Lycoris aurea* with four mannose binding sites per monomer<sup>★</sup>

Jiwei Liu<sup>1</sup>, Xiaochao Xu<sup>1</sup>, Jinzhi Liu<sup>1</sup>, Jan Balzarini<sup>2</sup>, Yongtin Luo<sup>1</sup>, Yang Kong<sup>1</sup>, Jian Li<sup>1</sup>, Fang Chen<sup>1</sup>, Els Van Damme<sup>3</sup> and Jinku Bao<sup>1</sup>✉

<sup>1</sup>College of Life Sciences, Sichuan University, Chengdu, P. R. China; <sup>2</sup>Rega Institute for Medical Research, K. U. Leuven, Belgium; <sup>3</sup>Department of Molecular Biotechnology, Ghent University, Gent, Belgium

Received: 27 November, 2006; revised: 20 February, 2007; accepted: 05 March, 2007  
available on-line: 14 March, 2007

The mannose-binding agglutinin from bulbs of *Lycoris aurea* (LAA) agglutinates rabbit but not human erythrocytes. The molecular mass of the monomer in SDS/PAGE is 12 kDa while the apparent molecular mass in gel filtration is 48 kDa, indicating that LAA is a homotetramer. The full-length cDNA of LAA contains 683 bp with an open reading frame encoding a protomer of 162 amino-acid residues. Hydrophobic Cluster Analysis and molecular modeling of the 109-residue mature polypeptide suggested a similar secondary and tertiary structure to those of *Narcissus pseudonarcissus* agglutinin (NPA). Molecular docking revealed that, besides the three mannose-binding sites common among *Amaryllidaceae* lectins, LAA also contains a fourth unique mannose-binding site formed by a tryptophan cluster. The existence of four mannose-binding sites in each monomer of LAA is very unusual and has only been reported for NPA earlier.

**Keywords:** *Lycoris aurea* agglutinin, sequence alignment, mannose-binding lectin, molecular modeling and docking

### INTRODUCTION

Numerous plant species accumulate proteins possessing one or more domains that recognize and bind specific carbohydrates, with no catalytic properties. Since 1987, the description of the first monocot mannose-binding lectin (MBL) from snowdrop bulbs, more monocot MBLs have been isolated and cloned from various tissues of families *Alliaceae*, *Amaryllidaceae*, *Araceae*, etc. Evidence has accumulated that all monocot MBLs reported to date consist of subunits with highly homologous sequences and overall similar tertiary structures, which suggests the existence of a single superfamily of mannose-

binding proteins (Barre *et al.*, 1996; Van Damme *et al.*, 2000).

Hitherto, monocot MBLs have already attracted a lot of attention in different scientific fields because of their interesting biological properties, such as the suppressive effect on (retro)viruses and the toxic effect against insects and nematodes (Van Damme *et al.*, 1998). Recently, MBLs, isolated from several *Amaryllidaceae* species such as *Galanthus nivalis*, *Narcissus pseudonarcissus* and *Lycoris radiata* were shown to exhibit inhibitory effect on sap-sucking insects including aphids and planthoppers when tested in artificial diet assays. However, the major field of interest in MBLs is in biomedical research

<sup>★</sup>The nucleic acid sequence reported appears in the EMBI, GenBank and DDBJ Nucleotide Sequence Databases under the accession number AY763112.

✉Corresponding author: Jinku Bao, College of Life Sciences, Sichuan University, Chengdu, 610064, P. R. China; tel.: (86 28) 854 10672; e-mail: baojinku@yahoo.com.cn

**Abbreviations:** Fru, fructose; Gal, galactose; GalNAc, *N*-acetylgalactosamine; Glc, galactose; GlcNAc, *N*-acetylglucosamine; GNA, *Galanthus nivalis* agglutinin; HCA, hydrophobic cluster analysis; HIV, human immuno-deficiency virus; LAA, *Lycoris aurea* agglutinin; LRA, *Lycoris radiata* agglutinin; MBL, mannose-binding lectin; NHA, *Narcissus hybrid* cultivar agglutinin; NPA, *Narcissus pseudonarcissus* agglutinin; PCR, polymerase chain reaction; RACE, rapid amplification of cDNA ends; SDS/PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; ZGA, *Zephyranthes grandiflora* agglutinin.

striving to exploit the potent anti-(retro) virus activity with the aim of developing new medicines such as microbicide drugs against HIV. Lectins from the *Orchidaceae* species, such as *Epipactis helleborine* agglutinin, have anti-RSV (respiratory syncytial virus) activity in HeLa cells, *Cymbidium hybrid* agglutinin has anti-CMV (anti-human cytomegalovirus) activity in HEL cells and *Listera ovata* agglutinin has anti-influenza virus activity in MDCK cells (Van Damme *et al.*, 1994). Moreover, these lectins are highly inhibitory to HIV-I/II in cell culture (Balzarini *et al.*, 1992). Also, lectins from the *Amaryllidaceae* species *Galanthus nivalis*, *Hippeastrum hybrid* and *Narcissus pseudonarcissus*, inhibit HIV-I/II infections at concentrations in the nanomolar range (Balzarini *et al.*, 2004). Furthermore, lectin from the *Amaryllidaceae* species *L. radiata* has been cloned and characterized as an MBL with three mannose-binding boxes, similar to other *Amaryllidaceae* lectins (Zhao *et al.*, 2003; Chang *et al.*, 2005). Extracts from bulbs of the closely related *L. aurea*, which is also a typical representative of the *Amaryllidaceae* and an important Chinese traditional herbal medicine, have been used as a treatment for skin herpesvirus in Chinese medicine for hundreds of years. Two isolectins have been obtained from the bulbs of *L. aurea* and characterized (Yagi *et al.*, 1993). Interestingly, an antiserum raised against these lectins reacted weakly with other lectins from the *Amaryllidaceae*, revealing differences in immunological reactivity and making the *L. aurea* isolectins exceptional among the *Amaryllidaceae* family lectins (Yagi *et al.*, 1993). Therefore, in this report, we focused on the purification, characterization and molecular cloning of the MBL from the bulbs of *L. aurea*. Molecular cloning of the LAA gene demonstrated that LAA should be considered as a novel member of the monocot MBLs. Molecular modeling and docking experiments showed that LAA is one of the very few lectins, like *N. pseudonarcissus* agglutinin (NPA), which have an additional mannose-binding site near the tryptophan cluster (Sauerborn *et al.*, 1999). This discovery extends our knowledge on protein-carbohydrate recognition, which is much more complex than other recognition processes such as protein-protein or protein-DNA interactions (Loris *et al.*, 2004), and justifies a more detailed evaluation of LAA for its biological (i.e. antiviral) properties.

## MATERIALS AND METHODS

**Plant material.** Young bulbs of *Lycoris aurea* collected from Sichuan province, China, in August (florescence), were instantly frozen in liquid nitrogen and preserved at  $-70^{\circ}\text{C}$  for RNA extraction.

**Protein determination.** Protein concentrations were determined as described by Lowry *et al.* (1951),

using crystalline bovine serum albumin as a standard.

**Extraction and purification of LAA.** Bulbs of *L. aurea* were homogenized in 0.14 mol/L NaCl in a Waring blender (Philips, Zhuhai, China) at  $4^{\circ}\text{C}$ . Then the homogenate was centrifuged ( $4000\times g$ , 10 min,  $4^{\circ}\text{C}$ ) and the supernatant was filtered through filter paper (Whatman 3MM, New Jersey, USA). Solid ammonium sulfate was added to the filtered extract at 80% saturation. The precipitate was collected by centrifugation ( $9000\times g$ , 30 min) and then dissolved and dialysed against 50 mmol/L Tris/HCl buffer (pH 7.4). After another centrifugation ( $9000\times g$ , 30 min,  $4^{\circ}\text{C}$ ) of the dialysate, the supernatant was subjected to fractionation on a DEAE-Sepharose Fast Flow column (Amersham Biosciences, Piscataway, USA) equilibrated with the same buffer. The bound protein was eluted with a linear gradient of 0–1.0 mol/L NaCl in the same buffer and active fractions were pooled and applied to gel filtration on a Sephacryl S-100 column equilibrated with 10 mmol/L sodium phosphate buffer (pH 7.4). After eluting the column with the same sodium phosphate buffer, the main peak with the strongest hemagglutinating activity was collected and lyophilized for further use.

**Determination of the molecular mass of LAA.** The molecular mass of LAA was estimated by gel filtration chromatography on the same Sephacryl S-100 column mentioned above. The column was calibrated with BSA (66.2 kDa), GNA (48 kDa), pepsin (35.5 kDa), and trypsin (23.3 kDa). Gel filtration-purified lectin was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS/PAGE), pH 8.3, using 12% separating and 5% stacking gels for subunit molecular mass determination (Laemmli, 1970).

**Hemagglutinating activity and saccharide inhibition assays.** Hemagglutinating activity was determined by the method of serial double-dilution. The sugar specificity was determined by comparing the inhibitory activity of hemagglutination in LAA solutions (25  $\mu\text{l}$ ) which were serially diluted with physiological saline and incubated with various concentrations of 80 mmol/L carbohydrates or 2% glycoprotein solution (25  $\mu\text{l}$ ) for 1 h. The carbohydrates and glycoproteins tested were: mannose, mannan, *N*-acetylgalactosamine, *N*-acetylglucosamine, galactose, fructose, glucose, sialic acid, ovalbumin, thyroglobulin.

**Amino-acid sequence analysis.** The  $\text{NH}_2$ -terminal amino-acid sequence of LAA was determined by automated Edman degradation on an Applied Biosystems model 476A sequencer (Applied Biosystems, Foster City, USA). Phenylthiohydantoin (PTH) amino acids were identified in a  $140^{\circ}\text{C}$  microgradient.

**RNA and DNA isolation.** Bulbs of *L. aurea* were powdered in liquid nitrogen with a mortar

and pestles. Total RNA and DNA were isolated using the RNA and DNA Extraction Kit (Takara Biotechnology Co. Ltd) according to the manufacturer's instructions.

**3'-RACE (rapid amplification of cDNA ends) and 5'-RACE of LAA gene.** cDNA synthesis was performed with the 3'-RACE System (GIBCO BRL, USA). Essentially, RNA was reversely transcribed with a cDNA synthesis primer (5' GGC CAC GCG TCG ACT AGT AC TTTTTTTTTTTTTTTTTTTT 3'). Degenerate primer 1 (5' TAC TCC/T GGC/T GAG ACT/C CTC TCT/C 3') was designed according to the N-terminal amino-acid sequences of LAA. PCR was performed (denaturing cDNA at 94°C for 5 min, then 29 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 2 min, and a final cycle at 72°C for 10 min). The PCR product was separated by electrophoresis on 1% agarose gel and purified and cloned into the pMD18-T vector for sequencing.

Based on the sequence of the 3'-RACE product, the specific primers 2 (5' CTG TGG TGC TGA TCC TGG AAG 3') and 3 (5' CCA ACG AGC GGT TCC GTA GAT 3') were designed to amplify the 5' end of LAA. According to the manufacturer's instructions of SMART™ RACE cDNA Amplification Kit, RNA was reversely followed by tailing cDNA with the SMART II oligonucleotide. The first round of PCR was performed with primer 2 and SMART III. The PCR product was diluted 50 fold for the second round of amplification with primer 3 and SMART III. The PCR product was purified and cloned into the pUC18-T vector for sequencing.

**Generation of full-length LAA cDNA sequence.** The sequences of the 3' and 5' cDNA ends were aligned to obtain a predicted full-length cDNA. According to the aligned sequence, a pair of specific primers (forward primer 4: 5' C AAA ATG GCT AAG ACA AGT TTC CTC 3' and reverse primer 5: 5' CTT CAT TAC ACA ACA TGC ATG C 3') was designed to amplify the LAA gene from genomic DNA. PCR was performed according to the conditions used in 3' RACE.

**Molecular modeling and docking.** Multiple amino-acid sequence alignment was performed using CLUSTAL W (Higgins *et al.*, 1994). Hydrophobic cluster analysis (Gaboriaud *et al.*, 1987; Lemesle-Varloot *et al.*, 1990) was performed to delineate the structurally conserved  $\beta$ -sheets along the amino-acid sequence of LAA using NPA as a model. Molecular modelling of LAA was carried out on Swiss-Model 3.5 (<http://swissmodel.expasy.org/>).

To investigate the docking mode of LAA, we selected the Lamarckian genetic algorithm (LGA) offered by AutoDock 3.0.5 (Solis & Wets, 1981; Morris *et al.*, 1998) in this study. The grid boxes centered on the selected amino acids (such as QDNYs, W41, etc.) include the entire binding site and provide

enough space for the ligand translational and rotational walk. Thirty runs were performed. For each independent run, a maximum number of 27000 LGA operations were generated on a population of 50 individuals. Operator weights for crossover, mutation, and elitism were default parameters (0.80, 0.02, and 1, respectively).

## RESULTS

### Purification and characterization of the LAA

The crude extract from bulbs of *L. aurea*, purified by ion exchange chromatography, yielded two adsorbed peaks (Fig. 1A), one (D1) devoid of hemagglutination activity and the other (D2) exhibiting strong hemagglutinating activity. D2, chromatographed on Sephacryl S-100 (Fig. 1B), yielded a very small peak (Q1) without a hemagglutinating activity and a large peak (Q2) with hemagglutinating activity. This purified lectin exhibited almost the same elution volume as GNA (Fig. 1C), the first described monocot mannose-binding lectin with a molecular mass of 48 kDa in gel filtration (Van Damme *et al.*, 1987) and a single band with a molecular mass of 12 kDa in SDS/PAGE (Fig. 2), indicating that LAA was a tetrameric protein with four identical subunits, which strikingly resembled other MBLs of *Amaryllidaceae* family.

LAA strongly agglutinated rabbit erythrocytes at a concentration of 0.49  $\mu\text{g}/\text{mL}$ , but did not agglutinate any type of human erythrocytes even at a concentration of 1 mg/ml. The hemagglutinating activity was specifically inhibited by mannan and thyroglobulin (Table 1). Mannose was able to partly inhibit the hemagglutinating activity. Other sugars did not inhibit the agglutinating activity of LAA.

### Cloning of the LAA gene

Based on thirty successfully sequenced NH<sub>2</sub> terminal amino acids (ENILYSGETLSPGDFLSYGSYVFIMQEDCN), primers were designed and RT-PCR and RACE were performed. As a result, a 516 bp fragment at the 3' end and a 411 bp fragment at the 5' end of LAA were obtained. Through analyzing the 3'-end and 5'-end sequence, the 683 bp full-length cDNA containing a 486 bp open reading frame and encoding a 162 amino-acid protein was obtained. Using the gene-specific primers 4 and 5, the full-length DNA and deduced amino-acid sequences were obtained by PCR on genomic DNA extracted from young bulbs of *L. aurea*. The nucleic acid sequence and the deduced amino-acid sequences from cDNA and genomic DNA proved

identical. This indicated that there was no intron in LAA. According to the rules of predicting the signal peptide (Jannick *et al.*, 2004) and in comparison with GNA for which the complete protein was sequenced (Van Damme *et al.*, 1991), a 23-amino acid-signal peptide before S23 and a 30-amino acid-C-

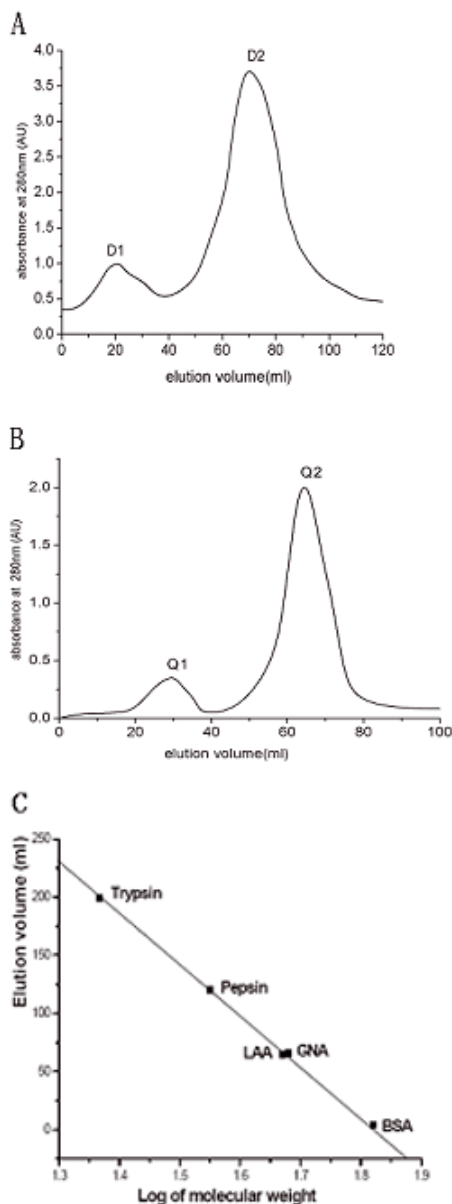
terminal cleavage peptide after H132 were identified. So, the mature polypeptide LAA contains 109 residues with a theoretic isoelectric point of 4.72 and a calculated molecular mass of 12.1 kDa, which is in good agreement with the molecular mass measured by SDS/PAGE.

### Homology analysis

Database retrieval with PSI-Blast (Altschul *et al.*, 1997) showed that LAA is a member of the monocot MBL superfamily. Multi-alignment of LAA with other monocot MBLs revealed that the sequence identity between LAA and *N. pseudonarcissus* agglutinin (NPA), *G. nivalis* agglutinin (GNA), *N. hybrid* cultivar agglutinin (NHA), *L. radiata* agglutinin (LRA) and *Zephyranthes grandiflora* agglutinin (ZGA) were 88.99%, 82.57%, 83.49%, 79.09% and 80.91%, respectively (Fig. 3). Like many other monocot MBLs, LAA monomer also has three mannose-binding sites (QDNY) (Fig. 3).

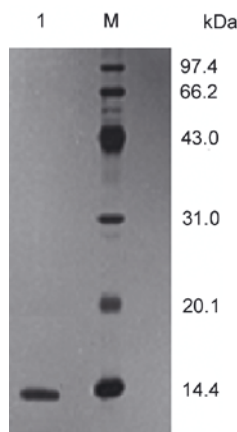
### Modeling and docking of LAA

Considering the highest identity (88.99%) between the amino-acid sequences, the coordinates of NPA (Sauerborn *et al.*, 1999) were selected as the model. Most of the buried residues of the polypeptides of both lectins are readily conserved (Fig. 3). A comparison of their HCA plots (Fig. 4) allowed an easy delineation along the amino-acid sequence of LAA of the twelve strands of the  $\beta$ -sheet present in NPA (Gaboriaud *et al.*, 1987; Lemesle-Varloot *et al.*, 1990). The three-dimensional model of LAA strongly resembles that of NPA (Fig. 5). Each subdomain contains a mannose-binding site of four conserved amino-acid residues (QDNY), which binds mannose through a network of four hydrogen bonds interconnecting the hydroxyls C2-OH, C3-OH and C4-OH of mannose to the four residues (Hester *et al.*, 1995; Hester & Wright, 1996; Sauerborn *et al.*, 1999). Furthermore, a valine residue, which interacts with C3



**Figure 1. Purification and molecular mass estimation of LAA.**

**A.** Elution profile of LAA on DEAE/Sepharose column pre-equilibrated with Tris/HCl buffer (pH 7.4). The bound protein was eluted with 0–1.0 mol/L NaCl gradient at a flow rate of 2 ml/min. **B.** Gel-filtration of active fraction D2 on Sephacryl S-100 column pre-equilibrated with sodium phosphate buffer (pH 7.4) at a flow rate of 45 ml/h. The elution profiles were monitored at 280 nm. **C.** Standards used for gel-filtration analysis were BSA (66.2 kDa), GNA (48 kDa), pepsin (35.5 kDa), and trypsin (23.3 kDa).



**Figure 2. SDS/PAGE analysis of purified LAA.**

Lane 1, purified LAA (30 µg). Lane M, molecular mass markers. The mass markers used were: rabbit phosphorylase B (97.4 kDa), bovine serum albumin (66.2 kDa), rabbit actin (43.0 kDa), bovine carbonic anhydrase (31.0 kDa), trypsin inhibitor (20.1 kDa), hen egg white lysozyme (14.4 kDa); the gels were stained with Coomassie Brilliant Blue.



Table 1. Inhibition of hemagglutination of LAA by carbohydrates and glycoproteins

Carbohydrates (80 mmol/L)	LAA concentration ( $\mu\text{g/mL}$ )										
	500	250	125	62.5	31.3	15.6	7.80	3.90	1.95	0.98	0.49
Control	+	+	+	+	+	+	+	+	+	+	+
Mannose	+	+	+	+	-	-	-	-	-	-	-
Mannan	-	-	-	-	-	-	-	-	-	-	-
Gal	+	+	+	+	+	+	+	+	+	+	-
GalNAc	+	+	+	+	+	+	+	+	+	+	-
Fru	+	+	+	+	+	+	+	+	+	+	-
Glc	+	+	+	+	+	+	+	+	+	+	-
GlcNAc	+	+	+	+	+	+	+	+	+	+	-
Sialic acid	+	+	+	+	+	+	+	+	-	-	-
Thyroglobulin (2%)	-	-	-	-	-	-	-	-	-	-	-
Ovalbumin (2%)	+	+	+	+	+	+	+	+	+	+	+

Note: +, agglutination; -, no agglutination

and C4 of mannose through van der Waals interactions, is also conserved in each subdomain of both NPA and LAA.

Interestingly, a fourth binding site observed in NPA may also exist in LAA (Fig. 5C). This unique site of NPA, using a tryptophan cluster to make contacts with carbohydrates, is difficult to describe in terms of sequence (Sauerborn *et al.*, 1999). As the docking experiment shows, the mannose molecule could have two alternative conformations (RMSD=5.6940 Å) (Fig. 5D). In the conformations  $\alpha$  and  $\beta$ , the binding energies are -3.81 kcal/mol and -3.37 kcal/mol, respectively, while the docking energies are -4.42 kcal/mol and -3.79 kcal/mol, respectively. In both conformations, mannose sits in a cleft on the LAA surface.

## DISCUSSION

This report describes a biochemical and molecular biological study of the MBL from *L. aurea*. Based on carbohydrate inhibition assays and analysis of the corresponding gene it was revealed that LAA belongs to the superfamily of monocot MBL. The agglutination reaction of LAA with various types of blood cells revealed that it can only agglutinate rabbit red blood cells but not human erythrocytes. Using protein purification techniques, we demonstrate that LAA is a tetrameric protein composed of four identical subunits of about 12 kDa. The two isolectins Yagi and coworkers purified in 1993 from Japanese *L. aurea* were both pure dimers which were both estimated to be 25 kDa by gel filtration and

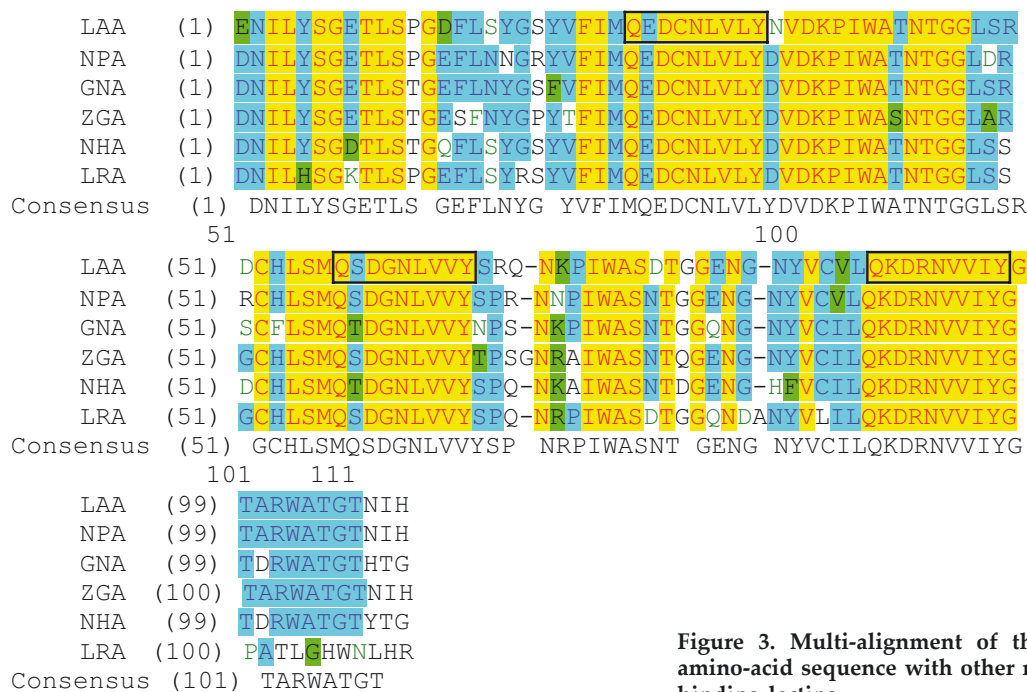
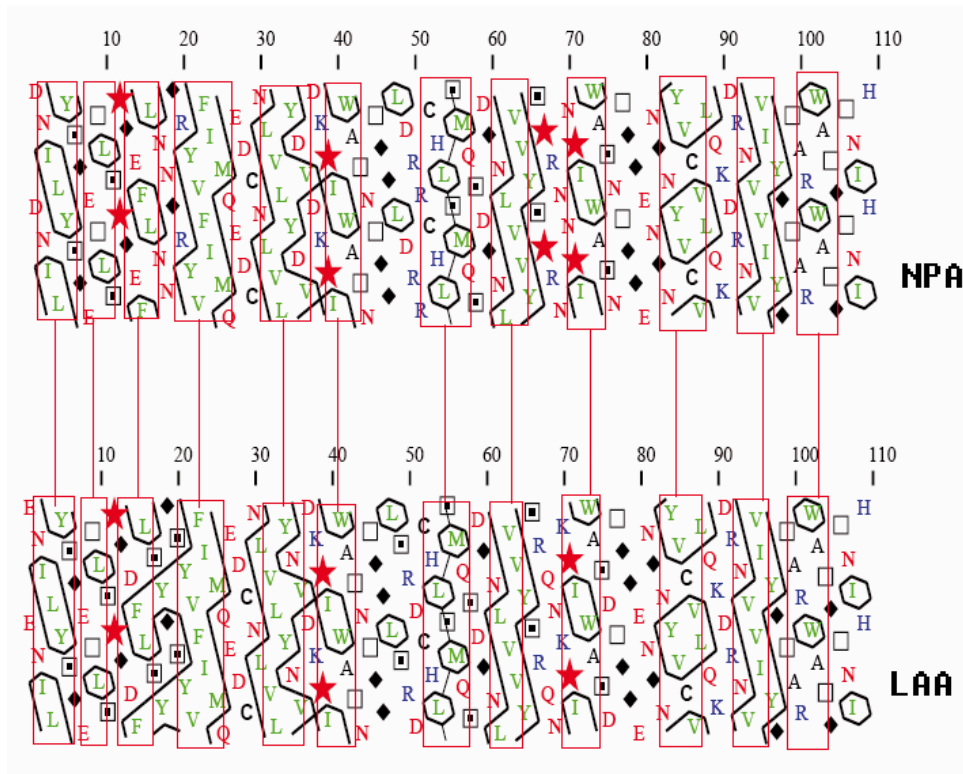


Figure 3. Multi-alignment of the predicted LAA amino-acid sequence with other monocot mannose-binding lectins. Mannose-binding sites (QDNY) are boxed.



**Figure 4. Comparison of predicted HCA plots of NPA (upper) and LAA (lower).**

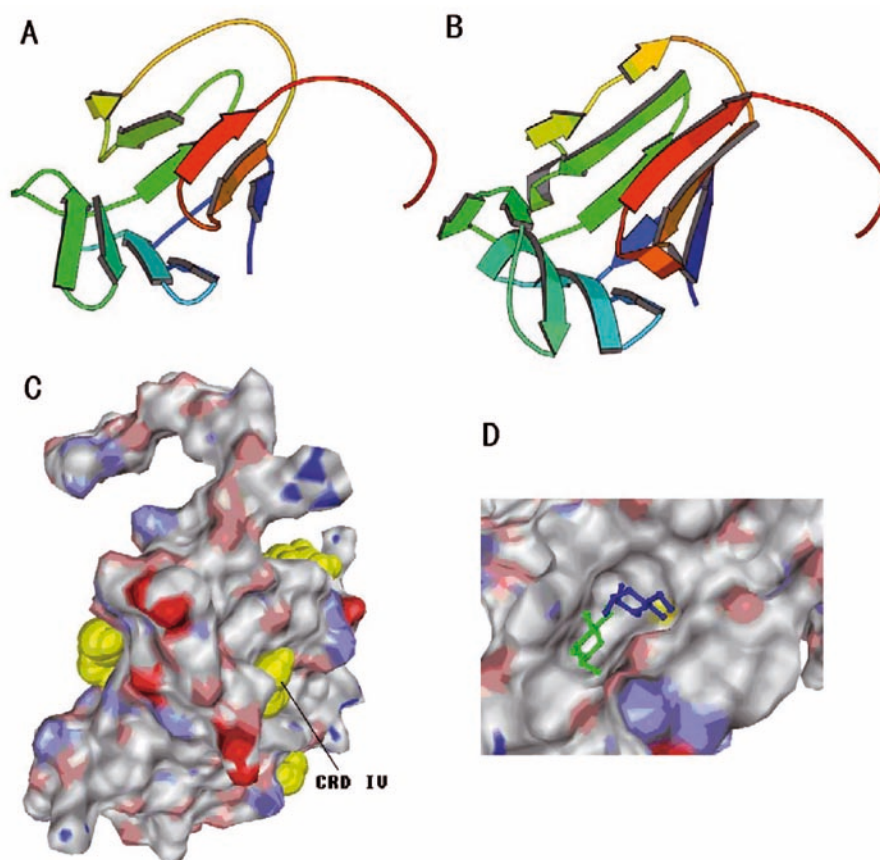
The structurally conserved regions which exhibit similar conserved hydrophobic properties between LAA and HCA are encircled in bold line.

12 kDa by SDS/PAGE. Only 15 amino acids at the N-terminus of the two dimers were sequenced, and we found that the fourteenth residue from the N-terminus of the subunit in our tetrameric protein was aspartic acid, whereas the dimeric counterpart had a glutamic acid. As no data is available about the full sequence of the two dimers of Yagi and coworkers no further sequence comparison between the dimers and the tetramer is feasible. It is possible that both the dimers and the tetramer are isolectins. The different environment between Kagoshima, Japan, and Chengdu, China may contribute to the differences between the lectins. The discovery of this new gene should encourage research in many fields, like the evolutionary relationships among the MBLs, the function of MBLs in plant physiology, and so on.

Analysis of the LAA sequence revealed the presence of three absolutely conserved mannose-binding sites (QDNY). Interestingly, the multiple alignment revealed the highest identity between LAA and NPA (88.99%) but not LRA, which, traditionally, is thought to be much closer to LAA in evolution. Using a homology model program, we obtained a three-dimensional structure of LAA which strongly resembles that of NPA. Surprisingly, further docking analysis showed a fourth carbohydrate-recognizing domain that existed near the tryptophan cluster and that might be involved in the recognition of naturally occurring glycans (Sauerborn *et al.*, 1999). As to our knowledge, LAA is the second monocot MBL discovered to date which has four

mannose-binding sites. Further structural research on LAA should enable discovering a new mode of interaction between mannose and such monocot MBLs.

It has been demonstrated that the gp120 envelope of HIV-1 contains approx. 50% of high-mannose-type oligosaccharides: all 24 N-linked glycosylation sites are utilized in HIV-1(III<sub>B</sub>) gp120; 11 contain hybrid and/or high-mannose-type structures while 13 of the glycosylation sites contain complex-type oligosaccharides. Balzarini *et al.* (1991; 2004) reported that the  $\alpha$ -(1-3)-D-mannose- and  $\alpha$ -(1-6)-D-mannose-specific agglutinins from *Galanthus nivalis*, *Hippeastrum hybrid*, *Narcissus pseudonarcissus* and *Listera ovata* inhibited infection of MT-4 and CEM cells by HIV I/II. Also, the mannose-binding lectin LRA, which is closely related to LAA, was found inhibitory against HIV-1 and HIV-2 at 0.29–0.43 and 0.59–0.60  $\mu$ g/ml, respectively, in MT-4 and CEM cell cultures, being virtually non-toxic at 100  $\mu$ g/ml (An *et al.*, 2006). López *et al.* (2003) reported an anti-HIV-1 activity of mannose-specific lectins from *Narcissus* species and showed that the anti-HIV activity of *N. tortifolius* lectin was 10- (in  $\mu$ g/mL) and 100- (in molarity)-fold more potent than that of the polyanion dextran sulfate, an HIV entry inhibitor. Thus, plant lectins may be considered as most interesting agents that potently inhibit HIV entry into its target cells. In addition, LAA exhibits no significant cytotoxicity towards T-lymphocyte CEM cells and simian kidney Vero cells (unpublished data). Therefore, it now seems imperative to evaluate LAA for its antiviral potential and to compare its antiviral activity



**Figure 5. Tertiary structure of LAA and its mannose-binding sites.**

**A.** Structure of NPA monomer. **B.** Structure of LAA monomer. **C.** The molecular surface of LAA monomer and its binding to mannose. **D.** The CRD (carbohydrate recognizing domain) IV and the binding to mannose, mannose in conformation  $\alpha$  is green and in conformation  $\beta$  is blue.

(not limited to HIV, but including other enveloped viruses) with those mannose-specific lectins that do not contain the fourth mannose-binding site in the monomers.

#### Acknowledgements

This work was supported by grants from the National Natural Sciences Foundation of China (No. 30000032 and 30270331). This work was also supported by the European Commission (EMPRO) and the Centers of Excellence of the K. U. Leuven (EF/05/15) (to J.B.).

#### REFERENCE

- Altschul SF, Madden TL, Schaffer AA, Zhang J, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**: 3389–3402.
- An J, Liu J-z, Wu C-f, Li J, Dai L, Van Damme E, Balzarini J, De Clercq E, Chen F, Bao J-k (2006) Anti-HIV I/II activity and molecular cloning of a novel mannose/sialic acid-binding lectin from rhizome of *Polygonatum cyrtoneuma* Hua. *Acta Biochim Bioph Sinica* **38**: 70–78.
- Balzarini J, Schols D, Neyts J, Van Damme E, Peumans W, De Clercq E (1991)  $\alpha$ -(1–3)- and  $\alpha$ -(1–6)-D-mannose-specific plant lectins are markedly inhibitory to human immunodeficiency virus and cytomegalovirus infections *in vitro*. *Antimicrob Agents Chemother* **35**: 410–416.
- Balzarini J, Neyts J, Schols D, Hosoya M, Van Damme E, Peumans W, De Clercq E (1992) The mannose-specific plant lectins from *Cymbidium hybrid* and *Epipactis helleborine* and the (*N*-acetylglucosamine)*n*-specific plant lectin from *Urtica dioica* are potent and selective inhibitors of human immunodeficiency virus and cytomegalovirus replication *in vitro*. *Antiviral Res* **18**: 191–207.
- Balzarini J, Hatse S, Vermeire K, Princen K, Aquaro S, Perno C-F, De Clercq E, Egberink H, Vanden Mooter G, Peumans W, Vandamme E, Schols D (2004) Mannose-specific plant lectins from the *Amaryllidaceae* family qualify as efficient microbicides for prevention of human immunodeficiency virus infection. *Antimicrob Agents Chemother* **48**: 3858–3870.
- Barre A, Van Damme EJM, Peumans WJ, Rougé P (1996) Structure-function relationship of monocot mannose-binding lectins. *Plant Physiol* **112**: 1531–1540.
- Bendtsen JD, Nielsen H, von Heijne G, Brunak S (2004) Improved prediction of signal peptides: SignalP 3.0. *J Mol Biol* **340**: 783–795.
- Chang LQ, Wu CF, LU HZ, Liu C, Gu Y, Chen F, Wu QQ, Bao JK (2005) Purification and characterization of agglutinin from bulbs of *Lycoris radiate* (*Amaryllidaceae*). *Chinese journal of applied and environmental biology*. **11**: 164–167.
- Gaboriaud C, Bissery V, Benchetrit T, Mornon JP (1987) Hydrophobic cluster analysis: an efficient new way to compare and analyse amino acid sequences. *FEBS Lett* **224**: 149–155.
- Hester G, Kaku H, Goldstein IJ, Wright CS (1995) Structure of mannose-specific snowdrop (*Galanthus nivalis*)

- lectin is representative of a new plant lectin family. *Nat Struct Biol* **2**: 472–479.
- Hester G, Wright CS (1996) The mannose-specific bulb lectin from *Galanthus nivalis* (Snowdrop) binds mono- and dimannosides at distinct sites Structure analysis of refined complexes at 2.3 Å and 3.0 Å resolution. *J Mol Biol* **262**: 516–531.
- Higgins D, Thompson J, Gibson T, Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**: 4673–4680.
- Laemmli HK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T<sub>4</sub>. *Nature* **227**: 680–685.
- Lemesle-Varloot L, Hennissat B, Gaboriaud C, Bissery V, Morgat A, Morion JP (1990) Hydrophobic cluster analysis: procedure to derive structural and functional information from 2-D representation of protein sequences. *Biochimie* **72**: 555–574.
- Loris R, Van Walle I, De Greve H, Beeckmans S, Deboeck F, Wyns L, Bouckaert J (2004) Structural basis of oligomannose recognition by the *Pterocarpus angolensis* seed lectin. *J Mol Biol* **11**: 1227–1240.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275.
- López S, Armand-Úgon M, Bastida J, Viladomat F, Esté JA, Stewart D, Codina C (2003) Anti-human immunodeficiency virus type 1 (HIV-I) activity of lectins from *Narcissus* species. *Planta Med* **69**: 109–112.
- Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, Olson AJ (1998) Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. *J Comput Chem* **19**: 1639–1662.
- Sauerborn MK, Wright LM, Reynolds CD, Grossmann JG, Rizkallah PJ (1999) Insights into carbohydrate recognition by *Narcissus pseudonarcissus* lectin: the crystal structure at 2 Å resolution in complex with α1–3 mannanobiose. *J Mol Biol* **290**: 185–199.
- Solis FJ, Wets RJ-B (1981) Minimization by random search techniques. *Math Oper Res* **6**: 19–30.
- Van Damme EJM, Allen AK, Peumans WJ (1987) Isolation and characterization of a lectin with exclusive specificity towards mannose from snowdrop (*Galanthus nivalis*) bulbs. *FEBS Lett* **215**: 140–144.
- Van Damme EJM, De Clercq N, Claessens F, Hemschoote K, Peeters B, Peumans WJ (1991) Molecular cloning and characterization of multiple isoforms of the snowdrop (*Galanthus nivalis* L.) lectin. *Planta* **186**: 35–43.
- Van Damme EJM, Smeets K, Torrekens S, Van Leuven F, Peumans WJ (1994) Characterization and molecular cloning of the mannose-binding lectins from three *Orchidaceae* species: *Listera ovata*, *Epipactis helleborine* and *Cymbidium hybrid*. *Eur J Biochem* **221**: 769–777.
- Van Damme EJM, Peumans WJ, Barre A, Rougé P (1998) Plant lectins: A composite of several distinct families of structurally and evolutionary related proteins with diverse biological roles. *Crit Rev Plant Sci* **17**: 645–662.
- Van Damme EJ, Astoul C, Barre A, Rougé AP, Peumans WJ (2000) Cloning and characterization of a monocot mannose-binding lectin from *Crocus vernus* (family Iridaceae). *Eur J Biochem* **267**: 5067–5077.
- Yagi F, Noguchi S, Suzuki S, Tadera K, Goldstein IJ (1993) Purification and characterization of isolectins from *Lycoris aurea*. *Plant Cell Physiol* **34**: 1267–1274.
- Zhao X, Yao J, Sun X, Tang K (2003) Molecular cloning and characterization of a novel lectin gene from *Lycoris radiata*. *DNA Seq* **14**: 223–226.