cDNA cloning and characterization of a mannose-binding lectin from *Zingiber officinale* Roscoe (ginger) rhizomes

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Using RNA extracted from *Zingiber officinale* rhizomes and primers designed according to the conservative regions of monocot mannose-binding lectins, the full-length cDNA of *Z. officinale* agglutinin (ZOA) was cloned by rapid amplification of cDNA ends (RACE). The full-length cDNA of *zoa* was 746 bp and contained a 510 bp open reading frame (ORF) encoding a lectin precursor of 169 amino acids with a signal peptide. ZOA was a mannose-binding lectin with three typical mannose-binding sites (QDNY). Semi-quantitative RT-PCR analysis revealed that *zoa* expressed in all the tested tissues of *Z. officinale* including leaf, root and rhizome, suggesting it to be a constitutively expressing form. ZOA protein was successfully expressed in *Escherichia coli* with the molecular weight expected. To our knowledge, this is the first mannose-binding lectin cDNA cloned from the family Zingiberaceae. Our results demonstrate that monocot mannose-binding lectins also occur within the family Zingiberaceae.

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1. Introduction

Lectins or agglutinins are a group of non-immunogenic proteins possessing at least one non-catalytic domain that binds reversibly to specific mono- or oligo-saccharide (glycoconjugate) (Peumans and Van Damme 1995). They are widespread throughout the plant kingdom occurring in a number of plant species from all major taxonomic groupings (Van Damme *et al* 1998). One such group is the so-called monocot mannose-binding lectins. Mannose-binding lectins are widely distributed in higher plants and are believed to play a role in recognition of high-mannose type glycans of foreign microorganisms or plant predators (Barre *et al* 2001). Up to now, monocot mannose-binding lectins have been cloned and characterized from seven families of angiosperms including Amarylli-

Keywords. Mannose-binding lectin; RACE; *Zingiber officinale*; ZOA

Abbreviations used: AAA, *Allium ascalonicum* agglutinin; ACPA, *Ananas comosus* (pineapple) agglutinin; AKA, *Amorphophallus konjac* agglutinin; APA, *Allium porrum* agglutinin; ASA, *Allium sativum* agglutinin; AUA, *Allium ursinum* agglutinin; CAA, *Crinum asiaticum* agglutinin; CHA, *Cymbidium hybrid* agglutinin; EHA, *Epipactis helleborine* agglutinin; GEAFP, *Gastrodia elata* antifungal protein; GNA, *Galanthus nivalis* agglutinin; LOA, *Listera ovata* agglutinin; NHA, *Narcissus hybrid* agglutinin; ORF, open reading frame; PCR, polymerase chain reaction; RACE, rapid amplification of cDNA ends; RT-PCR, reverse transcriptase-polymerase chain rection; TDA, *Typhonium divaricatum* agglutinin; TMA, *Taxus media* agglutinin; UTR, untranslated region; ZGA, *Zephyrathes grandiflora* agglutinin; ZOA, *Zingiber officinale* agglutinin.

daceae, Araceae, Alliaceae, Orchidaceae, Liliaceae, Iridaceae and Bromeliaceae (Van Damme *et al* 1991, 1993, 1994, 2000; Kai *et al* 2003) and from one family of gymnosperm (*Taxaceae*) discovered recently (Kai *et al* 2004), among which lectins from Amaryllidaceae species are most extensively studied and well documented (Van Damme *et al* 1998; Kai *et al* 2003).

In the past few years an increasing interest is drawn to this group of agglutinins for some reasons. One is because of their unique and exclusive specificities towards mannose and these lectins have become very interesting tools in glycoconjugate research (Haselbeck *et al* 1990). The other is the recent discovery that the snowdrop lectin exhibits striking toxicity to sap-sucking insects (Hilder *et al* 1995), which has provided the hope for insect control.

Zingiber officinale, commonly called ginger, is one of the traditional Chinese medicinal plant species and belongs to family Zingiberaceae. Together with *Allium sativum*, *Z. officinale* has been used in medicine and in flavouring foods. Crude ginger is used as an anti-emetic and expectorant, an anti-tussive and accelerator of the digestive organs. Semi-dried old crude ginger is also used for stomachache, chest pain, low back pain, cough, common cold and as a cure for a form of edema being called 'stagnate of water'. In the past few years mannose-binding lectins have been detected in the leaves and roots of garlic (*A. sativum*). Therefore it is worthwhile to investigate the presence of mannose-binding lectins in ginger, which will be helpful to study further the role of mannose-binding lectins in family Zingiberaceae. To date, there is no report on the cloning of mannose-binding lectin genes or cDNAs from family Zingiberaceae including *Z. officinale*.

In this paper, we report for the first time the molecular cloning and characterization of a novel full-length mannose-binding lectin cDNA from *Z. officinale*, which is also the first mannose-binding lectin cDNA isolated from family Zingiberaceae. The expression of *Zingiber officinale* in different tissues of *Z. officinale* and in *Escherichia coli* was also studied.

2. Materials and methods

2.1 *Plant materials*

Z. officinale plants were collected from the Second Military Medical University, China.

2.2 *RNA extraction*

The rhizome from *Z. officinale*, served as the starting material for RNA isolation, was powdered in liquid nitrogen with mortar and pestle and the total RNA was extracted using TRIzol Reagent (GIBCO BRL, USA) according to the manufacturer's instruction.

2.3 3′ *rapid amplification of cDNA ends*

cDNA synthesis was performed with the 3′ RACE System for rapid amplification of cDNA ends (RACE PCR Kit, GIBCO BRL, USA). Essentially, an aliquot of isolated RNA (approximately 2·5 μg) was reversely transcribed with a cDNA synthesis primer AP (adaptor primer, 5′-GGCCACGCGTCGACTAGTAC$(T)_{16}$-3′). As most of the monocot mannose-binding lectins have conserved mannose-binding sites with the conserved amino acid sequence of MQGDCNL, primer J001 (5′-ATGCAGGGCGACTGCAACCT-3′) was designed according to the conserved amino acid sequence and synthesized (Shanghai Sangon Biological Engineering Technology and Service Co. Ltd., People's Republic of China). The 3′ RACE was performed essentially according to the manufacturer's instructions. Reverse transcription (RT)-polymerase chain reaction (PCR) was conducted in terms of kit protocol except that the RT step was prolonged for a further reaction at 50°C for 30 min in case of a high GC content and complex secondary structure. PCR was carried out in a total volume of 50 μl containing 2 μl cDNA, 10 pmol each of J001 and AP primers, 10 μmol dNTPs, 1 × cDNA reaction buffer and 2·5U *Taq* polymerase. The PCR reaction was performed under the following conditions: cDNA was denatured at 94°C for 3 min followed by 35 cycles of amplification (94°C for 45 s, 57°C for 1 min, 72°C for 1 min) and by 72°C for 10 min. After agarose gel electrophoresis, the target DNA band was purified using Gel Extraction Mini Kit (Watson Biotechnologies, Inc., PR China), ligated to pGEM-T Easy vector (Promega, USA) and then transformed into *E. coli* strain DH5*a*. Competent cells were prepared using $CaCl_2$ double suspension method while hot-shock method was adopted in *E. coli* transformation in terms of the protocol of Sambrook *et al* (1989). The PCR positive clone was sequenced using T7/SP6 primers (Sangon).

2.4 5′ *RACE*

The 5′ RACE System for RACE (GIBCO BRL, Life Technologies, USA) was used for 5′ cDNA cloning. According to the sequencing result of 3′ cDNA end, two primers were designed and synthesized for 5′ RACE of *zoa*. RT, dC tailing and PCR amplifications were conducted in terms of the kit protocol with minor modification. The 5 μg total RNA extracted from leaves was reversely transcribed using primer JR-1 (5′-CCTCGCTTCTCACTCG-TTTTATTTG-3′) with an extra 30 min of RT at 50°C after standard RT at 42°C. Primers JR-1 and AAP (5′-GGCCACGCGTCGACTAGTACGGGIIGGGIIGGGIIG-3′) were used for primary amplification using 5 μl dC-tailed cDNA as the template in a total volume of 50 μl reaction system under the following PCR conditions:

cDNA was denatured at 94°C for 3 min followed by 30 cycles of amplification (94°C for 1 min, 55°C for 1 min, 72°C for 1 min) and by 72°C for 10 min. After checked by agarose gel electrophoresis, 0·1 µl of the PCR product was used as the template for nested PCR amplification using primers JR-2 (5′-ACCACGACGCCTTTGGAG-TTGG-3′) and AUAP under the following PCR conditions: 94°C for 3 min followed by 35 cycles of amplification (94°C for 1 min, 58°C for 1 min, 72°C for 1 min) and by 72°C for 10 min. The PCR product was subjected to electrophoresis, gel extraction, ligation, *E. coli* transformation and sequencing as mentioned above.

2.5 *Generation of* zoa *full-length cDNA sequence*

By comparing and aligning the sequences of 3′ and 5′ RACE products, the full-length cDNA sequence of *Z. officinale* lectin was deduced and obtained through RT-PCR reaction using primer JFL (5′-AGCATGGCTGGCC-TTGTCATC-3′) and AUAP. PCR was carried out in a total volume of 50 µl reaction solution containing 5 µl 10 × pfu buffer (plus Mg^{2+}), 1 µl 10 mM each of dNTPs, 1 µl 10 µM JFL, 1 µl 10 µM AUAP, 2 µl cDNA (3′ RACE product) and 2·5 units pfu DNA polymerase (Sangon) using the following protocol: 94°C for 3 min followed by 35 cycles of amplification (94°C for 1 min, 58°C for 1 min, 72°C for 1 min) and by 72°C for 10 min. The amplified blunt-ended DNA fragment was dA-tailed using a dA-tailing Kit (Sangon). DNA ligation with pGEM-T Easy vector, transformation of DH5*a* and sequencing were carried out as mentioned above.

2.6 *Computer analysis*

The encoded amino acid sequence of ZOA was deduced with DNA tools 5·0. The analysis and comparison of the deduced amino acid sequence of ZOA with other mannose-binding lectins published were performed using programs of blastp (Standard Protein-Protein BLAST) and blastn (Standard Nucleotide-Nucleotide BLAST) on NCBI (www.ncbi.nlm.nih.gov) and Vector NTI Suite 6·0.

2.7 *Semi-quantitative RT-PCR*

In order to investigate the expression pattern of *zoa* in different tissues including leaves, roots and rhizomes of *Z. officinale*, semi-quantitative one-step RT-PCR was carried out according to the manufacturer's instruction (Takara, Japan). Aliquots of total RNA (0·5 µg) extracted individually from leaves, roots and rhizomes of *Z. officinale* were used as templates in one-step RT-PCR reaction with two primers specific to coding sequence of *zoa* using one-step RNA PCR kit. Meanwhile, the RT-PCR reaction for the house-keeping gene (18S gene) using specific primers 18SF (5′-ATGATAACTCGACGGAT-CGC-3′) and 18SR (5′-CTTGGATGTGGTAGCCGTTT-3′) designed according to the conserved regions of plant 18S genes was performed as an internal control to estimate whether equal amounts of RNA among samples were used in semi-quantitative RT-PCR. Amplifications were performed under the following condition: 50°C for 30 min followed by 20 cycles of amplification (94°C for 30 s, 58°C for 30 s and 72°C for 30 s). The amplified products were separated on a 1% agarose gel and analysed with Gene analysis software package (Gene Company, USA). The RT-PCR amplification was performed three times.

2.8 *Expression of ZOA in* E. coli

The core fragment corresponding to ZOA mature protein was generated by PCR amplification using a pair of primers zoaup (5′-ATGGATCCGACAACGTTCTCTACTCTGG CGAC-3′) containing a *Bam*HI restriction site (underlined) and zoadp (5′-ATGAGCTCTTAATTATTGATC-ACCATGGCGATC-3′) containing a *Sac*I restriction site (underlined). After digestion with *Bam*HI and *Sac*I, the PCR product (the fragment corresponding to ZOA mature protein) was inserted into pQE30 vector pre-digested with *Bam*HI and *Sac*I. The resulting recombinant plasmid pQE30-*zoa* was sequenced to confirm the correct insert of *zoa* and transformed into *E. coli* M15 strain. M15 cell strains transformed with pQE30-*zoa* were grown in LB (Luria–Bertani) medium containing 100 µg/ml ampicillin and 25 µg/ml kanamycin at 37°C to an absorbance of 0·5–1·0 at 600 nm. Then the culture was induced by addition of 1 mM IPTG. The cells were harvested 3 h after induction and centrifuged at 4°C at 5000 rpm for 10 min. Some of the pellets were used to determinate the recombinant ZOA protein solubility according to the QIAexpressionist protocol. Subsequently, other pellets were resolved in pH 8·0 buffer B containing 10 mM Tris-HCl, 0·1 M NaH_2PO_4, and 8 M urea. Following centrifugation at 4°C at 15,000 rpm for 30 min, the supernatant was loaded into an equilibrated His-bond Ni Affinity Resin column (Watson, China), washed twice with buffer C (pH 6·3) and eluted twice with buffer E (pH 4·5). The eluates were collected respectively and all samples including the unbound fractions were analysed by SDS-PAGE. SDS-PAGE was performed as described by Laemmli (1970) using 4% and 12·5% polyacrylamide in the stacking and resolving gels, respectively, and proteins were detected with Coomassie blue staining.

2.9 *Haemagglutination assay*

Haemagglutination assays were carried out in round-bottomed microtitre plates. A total volume of 100 µl was

used in each well: 50 µl aliquot of serial two-fold dilutions of the lectin in 0·9% NaCl and 50 µl of 2% rabbit erythrocyte suspension. The microtitre plate with the 100 µl erythrocyte suspension per well, containing the serial double dilutions of the sample lectin with the final concentration being from 0 to 6·4 µg/ml, was incubated for 1 h at room temperature. Agglutination was assessed visually.

3. Results and discussion

3.1 *Cloning and sequence analysis of* zoa *full-length cDNA*

By 3′ and 5′ RACE, 3′ cDNA end of 578 bp and 5′ cDNA end of 301 bp were obtained respectively. By aligning and assembling these products the full-length cDNA of *zoa* was deduced, obtained by RT-PCR and further verified by sequencing. The full-length cDNA of *zoa* was 746 bp containing a 510 bp open reading frame (ORF) with 5′ untranslated region (UTR) of 34 bp upstream from the start codon and 3′ UTR of 202 bp downstream from the stop codon (figure 1). The 3′ UTR possessed typical low G + C content (40·59%) and one polyadenylation signal (AATAAA) was found within this region.

3.2 *Characterization of the predicted ZOA protein*

Zoa contains a 510 bp ORF with two possible initiation codons at positions 1 and 20 of the deduced amino acid sequence (figure 1). According to the criteria of Kozak (1981), the first initiation codon is most likely to be used since there is a purine at position – 3 and a G at position + 4 relative to the A of the respective ATG codon. Trans-

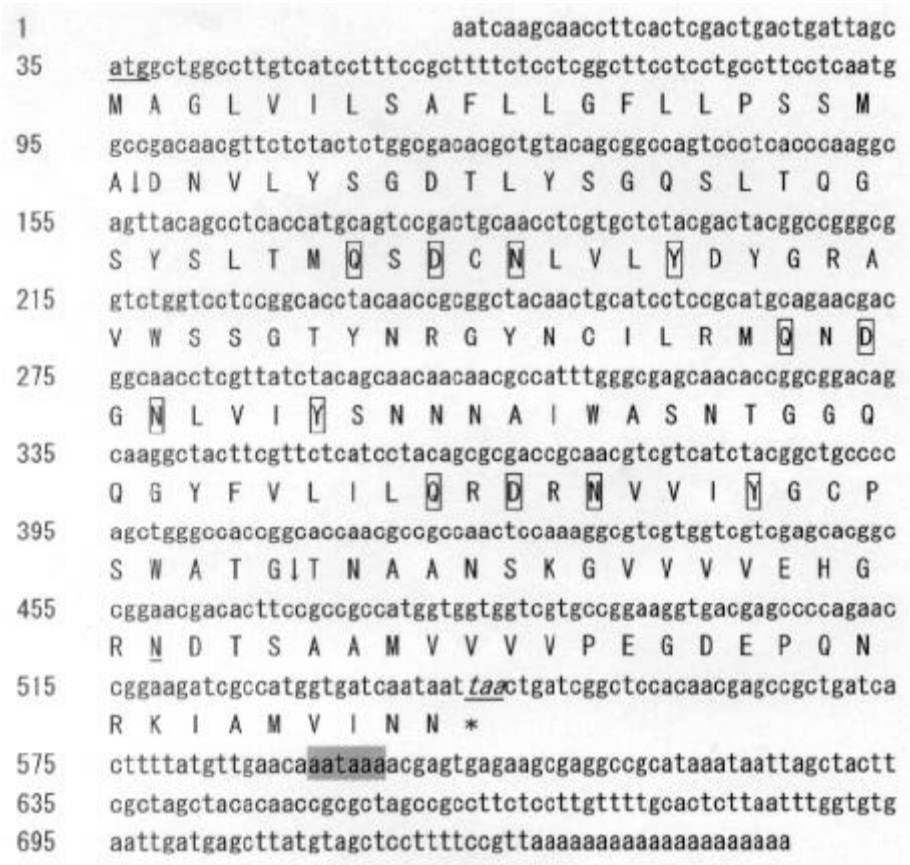

Figure 1. The full-length cDNA sequence and deduced amino acid sequence of ZOA. The start codon (atg) was underlined in bold and the stop codon (taa) was underlined in italics. Asterisk mark (*) represents that stop codon can not be translated into any amino acid. The signal for poly (A) tail-addition aataaa was painted with shadow. Mannose-binding sites (QDNY) were boxed. The putative processing sites for signal peptide sequence (between A and D) and the C-terminal extension (between G and T), were indicated by arrows. Putative glycosylation site (N) was underlined.

lation starting from the ATG codon yields a 169-amino-acid lectin precursor with a calculated molecular mass of 18·3 kDa. The ORF was translated with DNA tools 5·0, which also contains a signal peptide and a C-terminal peptide. According to the rule of predicting signal peptide (von Heijne 1986), the possible cleavage site of the signal peptide is between residues 21 and 22 of ZOA, resulting in a protein of 16·2 kDa.

The sequence, which is known to be the processing site for the cleavage of C-terminus of *G. nivalis* agglutinin, is also present in the C-terminal sequence of ZOA (Van Damme *et al* 1991). If Thr-Gly is also the processing site for the cleavage of the C-terminal sequence in ZOA, the cleavage at this site will result in lectin polypeptide of approximately 11·6 kDa. The hydrophobic characterization of this C-terminal peptide is consistent with the possibility that it is removed post-translationally. The loss of C-terminal extension has been reported in *Listera ovata* agglutinin (LOA) and *Cymbidium hybrid* agglutinin (CHA) (Van Damme *et al* 1994). However, the possible function of these C-terminal peptides remains unclear. *Zoa* encodes a precursor protein containing one potential glycosylation site within its C-terminal sequence.

Zoa encodes a mature lectin with three cysteins, different from previously reported Orchidaceae lectin LOA which has four cysteins, but is in good agreement with those of Araceae lectins such as *Amorphophallus konjac* agglutinin (AKA) (Fei *et al* 2003) and Orchidaceae lectins such as *Gastrodia elata* antifungal protein (GEAFP) and *Epipactis helleborine* agglutinin (EHA). The position of which coincides with the cysteins known to be involved in an internal disulfide bond in the previously cloned Amaryllidaceae lectin such as *G. nivali*s lectin. The third cystein was shown to occur as a free cystein in *G. nivali*s lectin (Van Damme *et al* 1991).

Sequences comparison by performing Blast Search in GenBank database revealed that ZOA had high homology to existing monocot mannose-binding lectins such as *Narcissus* hybrid, *Clivia miniata*, *G. nivalis*, *Ananas comosus*, *E. helleborine*, *Zephyranthes grandiflora*, *Allium ursinum*, suggesting that ZOA belonged to the monocot mannose-binding lectin superfamily. Multi-alignment of ZOA with other plant mannose-binding lectins revealed that ZOA was 59%, 49%, 61%, 56%, 55% and 58% identical to AKA (AY191004·1), *Allium ursinum* agglutinin (AUA) (U68531·1), CHA (U02516·1), GEAFP (AJ277786·1), *G. nivalis* agglutinin (GNA) (S19735), and *Narcissus hybrid* agglutinin (NHA) (AAA33546), respectively, with the corresponding similarity being 68%, 63%, 73%, 68%, 74% and 72% respectively (figure 2). ZOA also possessed three mannose-binding sites (QDNY; from Q_{47} to Y_{55}, Q_{78} to Y_{86} and Q_{109} to Y_{117} respectively), similar to many other monocot mannose-binding lectins (Van Damme *et al* 1991; Fei *et al* 2003; Kai *et al* 2003).

3.3 *Molecular evolution analysis*

To investigate the evolutionary relationship among different plant lectins, a phylogenetic tree was constructed based on the deduced amino acid sequences of ZOA and other plant lectins. All the monocot mannose-binding lectins were grouped into three big clusters (figure 3). The first cluster comprised ZOA, *Ananas comosus* (pineapple) agglutinin (ACPA), AKA, *Zephyrathes gradiflora* agglutinin (ZGA), *Crinum asiaticum* agglutinin (CAA), GEAFP, LOA, EHA, *Taxus media* agglutinin (TMA), *Typhonium divaricatum* agglutinin (TDA), AUA, *Allium porrum* (leek) agglutinin (APA), *Allium sativum* (garlic) agglutinin (ASA) and *Allium ascalonicum* (shallot) agglutinin (AAA), which seemed to be made up of several taxonomically unrelated families such as Alliaceae, Amaryllidaceae, Araceae, Bromeliaceae, Orchidaceae, Taxaceae and Zingiberaceae. As expected, the second cluster grouped most lectins of Amaryllidaceae family such as *Galanthus nivalis* and the third cluster contained most lectins from Araceae family such as *Pinellia ternata*, was in good agreement with the result reported previously (Kai *et al* 2004). All the above three groups of lectins were derived from a common ancestor in evolution, suggesting that these lectins shared a common evolutionary ancestor in plants.

3.4 *Semi-quantitative RT-PCR*

To examine the expression of *zoa* mRNA in various tissues, semi-quantitative RT-PCR analysis was performed. RT-PCR analysis revealed that *zoa* mRNA expression was detected in all tested tissues including root, rhizome and leaf, with the highest expression in rhizome figure 4). As *zoa* mRNA was detected in all the tested plant tissues, the *zoa* was considered to be a constitutively expressing one.

3.5 *Expression of recombinant ZOA in prokaryote*

Recombinant plasmid containing *zoa* was expressed in *E. coli* strain M15. SDS-PAGE showed that the molecular weight of His-tagged recombinant protein was about 17 kDa (figure 5). Due to the His-tag of around 1 kDa, the molecular weight of the ZOA protein is around 16 kDa, which is similar to that predicted by Vector NTI Suite 6·0.

3.6 *Haemagglutination assay*

The biological activity of recombinant ZOA was determined by haemagglutination assay. Recombinant ZOA agglutinated rabbit blood cells and the minimal concentration agglutinating rabbit erythrocytes was 2 μg/ml. The

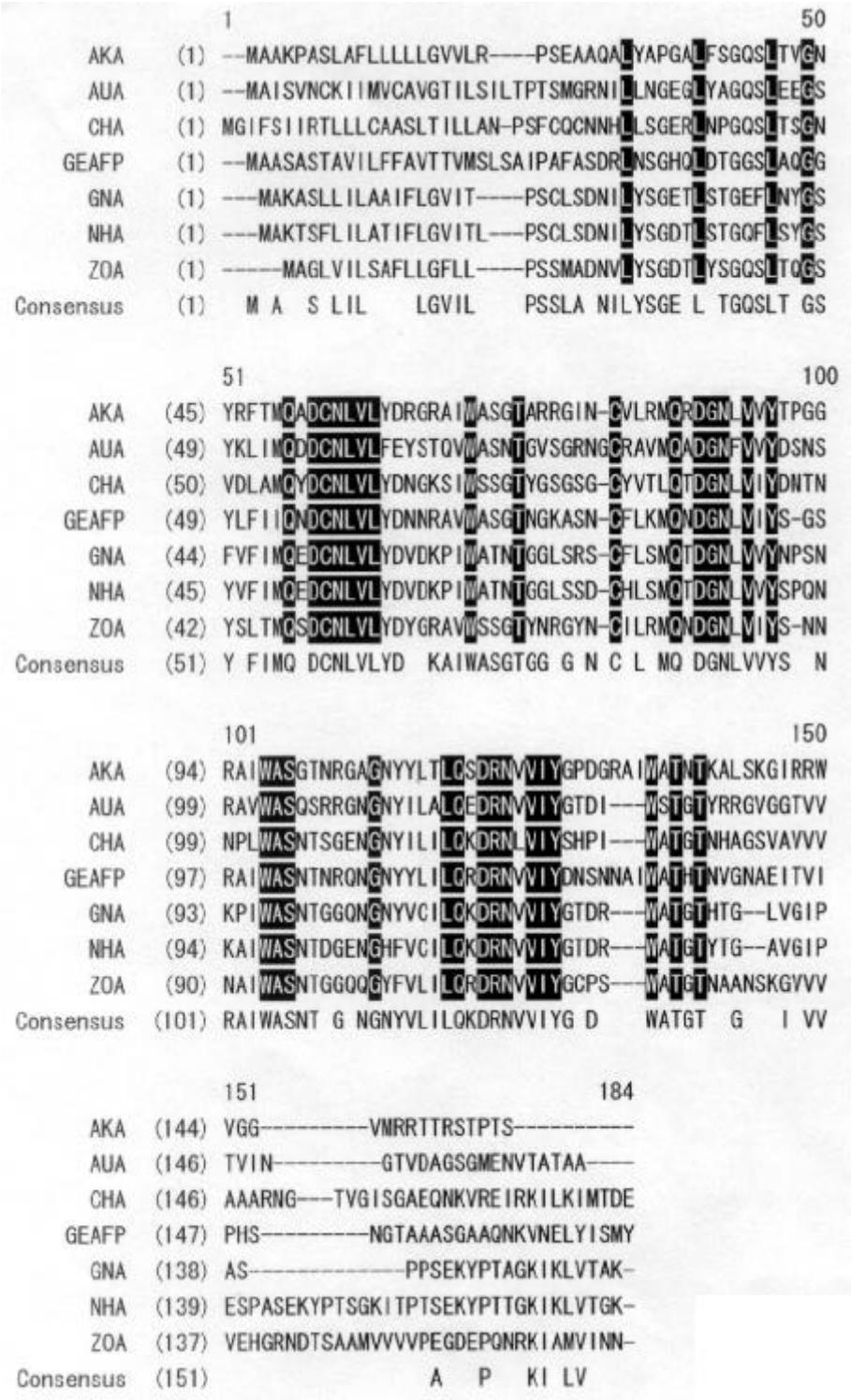

Figure 2. Multiple alignment of ZOA with other monocot mannose-binding lectins or agglutinins. AKA (AY191004·1); AUA (U68531·1); CHA (U02516·1); GEAFP (AJ277786·1); GNA (S19735); NHA (AAA33546). Gaps were introduced for optimal alignment and maximum similarity. Identical amino acids among all the aligned sequences were shown in black background.

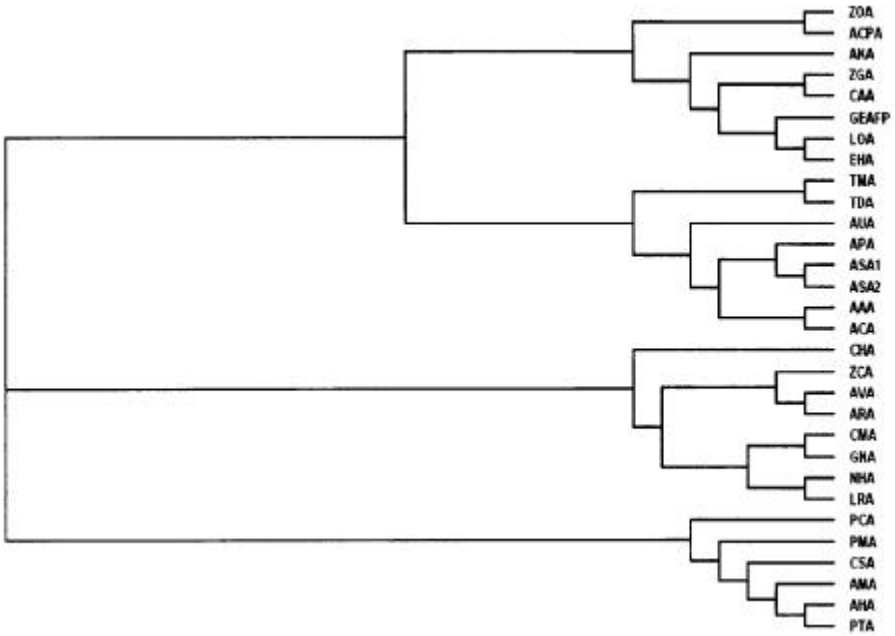

Figure 3. Phylogenetic tree analysis of ZOA and other mannose-binding lectins. ASA1 (AAB64237); ASA2 (AAB64238); AKA (AAP04617); APA (AAC37361); AAA (S39488); *Allium cepa* (onion) agglutinin (ACA, S39487); AUA (AAA16280); ACPA (AAM28277); *Arisaema heterophyllum* agglutinin (AHA, AAP50524); *Arum maculatum* (cuckoopint) agglutinin (AMA, AAC48998); *Amaryllis vittata* agglutinin (AVA, AAP57409); CHA (S43463); *Clivia miniata* agglutinin (CMA, AAA19910); *Crocus sativus* agglutinin (CSA, AAK29077); CAA (AAO59506); EHA (AAC48927); GNA (AAA33349); GEAFP (CAB94239); LOA (AAC37423); *Lycoris radiata* agglutinin (LRA, AAP20877); NHA (AAA33546); *Polygonatum cyrtonema* agglutinin (PCA, AAM28644); *Polygonatum multiflorum* agglutinin (PMA, AAC49412); *Hippeastrum rutilum* agglutinin (HRA, AAN73326); *Pinellia ternata* agglutinin (PTA, AAP20876); TMA (AY563631); TDA (AAQ55289); ZGA (AAQ18904); *Zephyrathes candida* agglutinin (ZCA, AAM94381).

agglutination occurred after the recombinant ZOA was added to the rabbit erythrocytes. This is in agreement with GNA (Van Damme *et al* 1987).

To our knowledge, this is the first report of a mannose-binding lectin cDNA isolated from family Zingiberaceae. The isolated full-length cDNA of *Z. officinale* lectin shares typical features of monocot mannose-binding lectins such as conserved protein active sites and signal peptide, indicating that ZOA belongs to the superfamily of monocot mannose-binding lectins. Sequence comparison reveals that ZOA has high similarity to GNA (72%) which is the toxic to sap-sucking insects (Powell *et al* 1993) and to GEAFP (68%) which exhibits antifungal properties (Wang *et al* 2001), so it is speculated that ZOA may also have similar inhibitory effect on sap-sucking insects or fungi growth. The cloning of *zoa* will also enable us to test its potential functions for pest resistance by transferring it into rice in the future. Interestingly, molecular evolution analysis reveals that ZOA from Zingiberaceae is very homologous to the typical Orchidaceae lectins such as

Figure 4. Expression patterns of *zoa* in different tissues (leaf, root and rhizome) of *Z. officinale* revealed by semi-quantitative RT-PCR analysis. The 18s rRNA was used as internal control paralleling in RT-PCR.

LOA and EHA, which is similar to some newly discovered non-Orchidaceae lectins such as ZGA from Amaryllidaceae and even TMA from gymnosperm species Taxaceae (Kai *et al* 2004). Our results further support that mannose-binding lectins are widely distributed than previously believed and suggest that mannose-binding lectins own very important and conserved function in evolutionary history. The identification of the *Z. officinale* lectin as a new member of the monocot mannose-binding lectins extends the occurrence of this family to

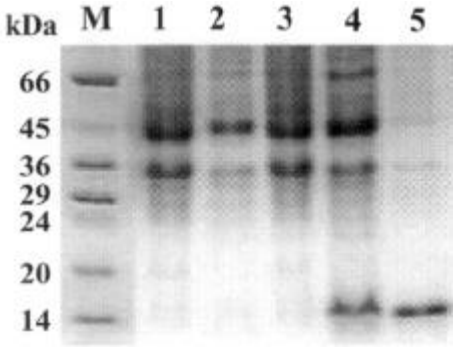

Figure 5. Expression of recombinant *zoa* in *E. coli* strain M15. M, molecular mass markers; lane 1, *E. coli* M15 containing no vector; lane 2, *E. coli* M15 containing pQE30; lane 3, *E. coli* M15 containing pQE30-ZOA not induced by IPTG; lane 4, *E. coli* M15 containing pQE30-ZOA induced by IPTG (1 mM); lane 5, recombinant ZOA protein purified from *E. coli*.

the *Zingiberaceae*, which also provides new insights into further understanding of the molecular evolution of monocot mannose-binding lectins.

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References

Barre A, Bourne Y, Van Damme E J M, Peumans W J and Rouge P 2001 Mannose-binding plant lectins: different structural scaffolds for a common sugar-recognition process; *Biochimie* **83** 645–651

Fei J, Liao Z H, Chai Y R, Pang Y Z, Yao J H, Sun X F and Tang K X 2003 Molecular cloning and characterization of a novel mannose-binding lectin gene from *Amorphophallus konjac*; *Mol. Biol. Rep.* **30** 177–183

Haselbeck A, Schickaneder E, Vondereltz H and Hosel W 1990 Structural characterization of glycoprotein carbohydrate chains by using digoxigenin-labeled lectins on blots; *Anal. Biochem.* **191** 25–30

Hilder V A, Powell K S, Gatehouse A M R and Gatehouse J A 1995 Expression of snowdrop lectin in transgenic tobacco plants results in added protection against aphids; *Transgenic Res.* **4** 18–425

Kai G Y, Zheng J G, Zhang L, Pang Y Z, Liao Z H, Li Z G, Zhao L X, Sun X F and Tang K X 2003 Molecular cloning of a new lectin gene from *Zephyrathes grandiflora*; *DNA Seq.* **14** 335–338

Kai G Y, Zhao L X, Zheng J G, Zhang L, Miao Z Q, Sun X F and Tang K X 2004 Molecular cloning and characterization of a new mannose-binding lectin gene from *Taxus media*; *J. Biosci.* **29** 399–407

Kazok M 1981 An analysis of 5′-noncoding sequence from 699 vertebrate messenger RNAs; *Nucleic Acids Res.* **9** 5233–5252

Laemmli U K 1970 Cleavage of structural proteins during the assembly of the head of bacteriophage T4; *Nature* (*London*) **227** 680

Peumans W J and Van Damme E J M 1995 Lectins as plant defense proteins; *Plant Physiol.* **109** 347–352

Powell K S, Gatehouse A M R, Hilder V A and Gatehouse J A 1993 Antimetabolic effects of plant-lectins and plant and fungal enzymes on the nymphal stages of 2 important rice pests, *Nilaparvata lugens* and *Nephotettix cinciteps*; *Entomol. Exp. Appl.* **66** 119–126

Sambrook J, Fritsch E F and Maniatis T 1989 *Molecular Cloning*: *A Laboratory Manual*, 2nd edition (New York: Cold Spring Harbor Laboratory Press)

Wang X C, Bauw G, Van Damme E J M, Peumans W J, Chen Z L, Van Montagu M, Angenon G and Dillen W 2001 Gastrodianin-like mannose-binding proteins: a novel class of plant proteins with antifungal properties; *Plant J.* **25** 651–661

Van Damme E J M, Allen A K and Peumans W J 1987 Isolation and characterization of a lectin with exclusive specificity towards mannose from snowdrop (*Galanthus nivalis*) bulbs; *FEBS Lett.* **215** 140–144

Van Damme E J M, Declercq N, Claessens F, Hemschoote K, Peeters B and Peumans W J 1991 Molecular-cloning and characterization of multiple isoforms of the snowdrop (*Galanthus-nivalis* L.) lectin; *Planta* **186** 35–43

Van Damme E J M, Smeets K, Engelborghs I, Aelbers H, Balzarini J, Pusztai A, van Leuven F, Goldstein I J and Peumans W J 1993 Cloning and characterization of the lectin cDNA clones from onion, shallot and leek; *Plant Mol. Biol.* **23** 365–376

Van Damme E J M, Smeets K, Torrekens S, Van Leuven F and Peumans W J 1994 Characterization and molecular cloning of mannose-binding lectins from the *Orchidaceae* species *Listera ovata*, *Epipactis helleborine* and *Cymbidium* hybrid; *Eur. J. Biochem.* **221** 769–777

Van Damme E J M, Peumans W J, Barre B and Rouge 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** 1–112

Van Damme E J M, Astoul C H, Barre A, Rougé P and Peumans W J 2000 Cloning and characterization of a monocot mannose-binding lectin from *Crocus vernus* (family Iridaceae); *Eur. J. Biochem.* **267** 5067–5077

von Heijne G 1986 A new method for predicting signal sequence cleavage sites; *Nucleic Acids Res.* **14** 4683–4690

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