Cloning and expression of a novel cDNA encoding a mannose-specific jacalin-related lectin from *Oryza sativa*

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Abstract

Lectin plays an important role in defense signaling in plants. A few genes of this family have been cloned. Here we report on a mannose-specific jacalin-related lectin in rice. Using sequence information of wheat gene VER2, which we had previously cloned, we were able to amplify a cDNA of *OsJAC1* from *Oryza sativa* by RT-PCR. The cDNA of *OsJAC1* was 1172 bp and contained a 921-bp open reading frame (ORF) encoding dirigent (amino acids 26–139) and jacalin (amino acids 175–305) domains of 306 amino acids. Comparison of the *OsJAC1* sequence with those of other lectins (jacalin) from rice, wheat and other species revealed that *OsJAC1* had the 12 amino acid positions conserved in all mannose-binding lectins. Semi-quantitative RT-PCR revealed that *OsJAC1* expression was present in stems, leaves and young spikes but not young roots; the expression was high in leaves and low in stems and spikes. And methyl jasmonate could induce the expression of *OsJAC1*. To test the activity of *OsJAC1*, the jacalin domain at the C-terminal was expressed in *E. coli* BL21 (DE3). The purified recombinant protein could agglutinate red blood cells of rabbit, and the agglutination activity was strongly inhibited by mannose compared with other carbohydrates. These results indicate that lectin with dirigent and jacalin domains exists in rice as well as wheat. This is the first report of cDNA cloning of mannose-binding jacalin-related lectin with a dirigent domain in N-terminal region from *O. sativa*.

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1. Introduction

Carbohydrate-binding proteins, also called lectins or agglutinins, are a heterogeneous group of proteins classified together on the basis of their ability to bind in a reversible way to well-defined simple sugars and/or complex carbohydrates (Van Damme et al., 1998). They can be classified into seven families of structurally and evolutionary related proteins (amaranthins, Cucurbitaceae phloem lectins, lectins with hevein domain(s), jacalin-related lectins, legume lectins, monocot mannose-binding lectins and type-2 ribosome-inactivating proteins) (Van Damme et al., 1998; Murdock and Shade, 2002). The wide distribution of lectins in all tissues of plants and their ubiquitous presence in the plant kingdom suggest important roles for these proteins. One possible
physiological function that has emerged is the defensive role of these carbohydrate-binding proteins against biotic stress (phytopathogenic microorganisms, phytophagous insects and plant-eating animals) (Chrispeels and Raikhel, 1991; Gatehouse et al., 1995) and abiotic stresses such as salinity (Zhang et al., 2000).

In the past few years, increasing interest has focused on mannose-binding lectin as interesting tools in glycoconjugate research (Haselbeck et al., 1990). As well, mannose-binding lectins are believed to play a role in recognizing the high-mannose-type glycans of foreign microorganisms or plant predators (Barre et al., 2001), which has provided hope for insect control. A recent discovery is that the snowdrop lectin has a striking toxicity to sucking insects (Hilder et al., 1995; Nagadhara et al., 2004). The mannose-binding snowdrop lectin binds to the mid-gut of the brown plant hopper and the green leafhopper (Foissac et al., 2000), thus preventing nutrient absorption. Many monocot mannose-binding lectins from Amaryllidaceae, Alliaceae, Araceae, Iridaceae, Liliaceae and Orchidaceae have been documented and their protein properties and functions well characterized (Van Damme et al., 1991, 1993, 1994, 2000).

When plants are stimulated by specific biotic or abiotic stimuli, their response is the expression of cytoplasmic and/or nuclear plant lectins (Van Damme et al., 2004). Abiotic stress and biotic stress are major limiting factors in crop growth and grain yield. Wheat and rice are important food grains consumed directly by humans. Recently, some mannose-binding lectins in wheat were cloned and their potential functions in defense discussed. Wheat chemically induced gene 1 (Wei-1) showing high expression during systemic acquired resistance (Görlach et al., 1996) may be a plant protection member. Hessian fly-response gene 1 (Hf-r-1), encoding a jacalin-like mannose-binding lectin, showed increased mRNA levels when the leaves of wheat containing the H9 resistance gene responded to specific biotypes of the Hessian fly (Williams et al., 2002); the cDNA sequence was similar to that of a maize gene encoding a β-glucosidase aggregating factor (BGAF) (Blanchard and Ensen, 2000) and to Hv-JA1 (a barley gene that responds to jasmonate) (Lee et al., 1996). A jasmonate-regulated protein (JRP-32) related to BGAF, is also a mannose-binding lectin (Wang and Ma, 2005).

Protein structure analysis indicated that Wei-1, together with Hf-r-1, maize BGAF, and JRP-32, contain two functional domains: a disease-response domain and a jacalin-related lectin domain. But in rice, only 1 jacalin-related mannose-binding lectin, salT, without a disease-response domain, was isolated from salt-stressed plants, characterized and found to be involved in adaptation to adverse osmotic conditions (Garcia et al., 1998; Zhang et al., 2000). Using sequence information of wheat gene vernalization-related gene 2 (VER2) we previously had cloned (Yong et al., 2003), we cloned and expressed a rice cDNA encoding mannose-binding jacalin-related lectin (designated OsJAC1 in this paper) with a disease-response domain in the N-terminal region.

2. Materials and methods

2.1. Isolation of the OsJAC1 gene

Oryza sativa (cv. ZhongHua 10) plants were grown in a greenhouse under normal irrigation and fertilization. Total RNA for semi-quantitative RT-PCR was extracted using TRIZOL Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions from stems, leaves and young spikes of flowering plants and young roots of seedlings (14 days old). To see the effect of Jasmonate in levels of OsJAC1 expression, rice seedlings (14 days old) were immersed into 50 μM methyl jasmonate solution for some hours and then used to extract total RNA for semi-quantitative RT-PCR. The cDNA synthesis was performed using AMV reverse transcriptase (Promega, USA) according to the manufacturer’s protocol. The OsJAC1 gene was amplified with the forward primer OsJAC1F (5’-gattactgcagcgcataactca-3’) and the reverse primer OsJAC1R (5’-gatgcatgccactacagacgta-3’) according to the sequence of rice cDNA clone (AK066682). The forward primer (TubulinF, 5’-tactgactcccaactacaga-3’) and the reverse primer (TubulinR, 5’-ttggtgactcggcaacaga-3’) of rice tubulin were used to amplify tubulin as an internal standard for RT-PCR. The PCR reaction was performed under the following conditions: cDNA was denatured at 94 °C for 2 min followed by 23–30 cycles of amplification (94 °C for 30 s, 57 °C for 30 s, 72 °C for 1 min) and followed by 72 °C for 10 min. The PCR product was inserted in pGEM®-T Easy vector (Promega, USA) and sequenced.

2.2. Purification of the recombinant protein

To test the activity of OsJAC1, the jacalin domain at the C-terminal of OsJAC1 was expressed in E. coli BL21 (DE3) cells. The 3’-terminal sequence (438 bp) of the open reading frame (ORF) added with the start codon (ATG) was inserted into the pGEX4T-1 vector and expressed in E. coli. A promoter inducible by IPTG controls the production of the fusion protein in the pGEX expression system. The fragment was amplified by PCR with a pair of primers (5’ga agatctatgtcctgccctgaaag-3’) containing a BglII restriction site (underlined) and (5’ccg cttgag gatgctgctcagtc-3’) containing an XhoI restriction site (underlined). After digestion with BglII and XhoI, the amplified product was inserted into the pGEX4T-1 vector pre-digested with BamHI and XhoI. The resulting recombinant plasmid pGEX4T-1 OsJAC1 was sequenced and transformed into E. coli. Transformed BL21 cells were grown in 600 mL Luria-Bertani (LB) liquid
medium containing 100 mg/L ampicillin with vigorous agitation at 37°C. Isopropyl β-D-thiogalactoside (IPTG) was added to the medium at a final concentration of 1 mM after the OD 600 reached 0.8. The cells were harvested 3 h after induction and centrifuged at 4°C at 2180g for 10 min. The bacterial cells were re-suspended in lysis buffer (50 mM Tris–HCl, pH 8.0, 100 mM NaCl) with constant agitation for 20 min on ice. A total of 80 μL lysozyme (50 mg/mL) and 16 mg sodium deoxycholate were added during the agitation. Then DNase I and MgCl2 were added to a final concentration of 80 and 200 mM, respectively. The cell solution was centrifuged at 12,000g for 10 min. The supernatant was applied to the affinity matrix glutathione sepharose 4B column (Pharmacia) with a bed volume of 2 mL, which had previously been washed and equilibrated with 1× phosphate buffered saline (PBS: 140 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 1.8 mM KH₂PO₄, pH 7.3) overnight. The column was washed with 20 mL 1×PBS three times. The recombinant protein was incubated with elution buffer (50 mM Tris–HCl, pH 8.0) containing 6 mg reduced glutathione for 30 min at 4°C and eluted from the affinity column. The purified GST-OsJAC1 was analysed by 12% SDS-PAGE.

2.3. Agglutination activity and carbohydrate-binding property tests

Agglutination activity and carbohydrate-binding property tests were carried out according to a two-fold serial diluting procedure (Debray et al., 1981). The rabbit erythrocytes were washed with buffered saline (150 mM NaCl, 6.8 mM Na₂HPO₄, 31.6 mM NaH₂PO₄, pH 7.2) until the red color of the blood was not observed. Agglutination assays were carried out in a 96-U-well plate in a final volume of 40 μL containing 20 μL of the recombinant protein (1 mg/mL) solutions and 20 μL of the buffered saline. The solution was then serially diluted in two-fold increments. Then 20 μL of a 1% suspension of red blood cells in the buffered saline was added. Agglutination was assessed visually after 1 h at room temperature.

The carbohydrate-binding specificity of OsJAC1 was determined by the inhibition of agglutination of rabbit erythrocytes. A total of 20 μL of inhibitors (200 mM), including GlcNAc (N-acetyl-D-glucosamine), Gal (D-galactose), Glc (D-glucose), and Man (D-mannose) dissolved in buffered saline (150 mM NaCl, 6.8 mM Na₂HPO₄, 31.6 mM NaH₂PO₄, pH 7.2) was added into the 20 μL

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Fig. 1. cDNA sequence and deduced amino acid sequence of OsJAC1. (A) The start codon (ATG) and the stop codon (TAA) are highlighted in grey. (B) Putative conserved domains have been detected with the protein–protein BLAST tool of NCBI (http://www.ncbi.nlm.nih.gov).
Fig. 2. Sequence conservation of OsJAC1. (A) Alignment of the deduced amino acid sequences for OsJAC1 and sequences encoded by related genes. Multiple sequence alignment was constructed employing the software DNAMAN. Residues that are identical in the nine sequences are highlighted in gray and invariants in black. The 12 amino acid positions indicated by black triangles are conserved in all mannose-binding lectins (Bourne et al., 1999). Black circles indicate three regions of the jacalin-related lectins that bind mannose. (B) Dendrogram showing the phylogenetic relationships of OsJAC1 with these related proteins. The tree was constructed employing the alignment resulting from analysis by DNAMAN.
serially diluted solutions in a 96-U-well plate. After pre-incubation for 1 h at room temperature, the 20-µL suspension of rabbit erythrocytes was added to and the agglutination was evaluated after 1 h at room temperature.

3. Results and discussion

3.1. Isolation and sequence analysis of rice OsJAC1 clone

To isolate the jacalin gene OsJAC1 in rice, full-length cDNA was amplified by RT-PCR. The cDNA of OsJAC1 was 1172 bp long and contained a 921-bp ORF from 73 to 993 bp (Fig. 1(A)). The ORF encoded a protein consisting of 306 amino acids with a calculated Molecular mass of 33.2 kDa, with pI of 6.4. The 306 amino acids encode a dirigent (amino acids 26–139) and a jacalin (amino acids 175–305) domain according to a BLAST protein–protein search of NCBI (http://www.ncbi.nlm.nih.gov) (Fig. 1(B)).

Multi-alignment of OsJAC1 with other mannose-binding lectins was conducted. The deduced amino acid sequence of OsJAC1 was about 48% identical to sequences encoded by related genes: Jacalin (jackfruit, AAA32679), salT (O. sativa, Z25811), Ta-VER2 (Triticum aestivum, BAA32786), Hv-JA1 (jasmonate-induced gene, barley, AAA87042.1), BGAF (maize, AF232008), Ta-JA1 (jasmonate-induced gene, T. aestivum, AAR20919), Hfr-1 (T. aestivum, AAM46813), and Wci-1 (T. aestivum, T06273) (Fig. 2(A)).

Protein structure analysis indicated that OsJAC1, together with the other proteins, contains two functional domains: a disease-response domain and a jacalin-related lectin (JRL) domain, except for jacalin or salT with a jacalin-related lectin domain. The sequence similarity between these mannose-binding jacalin-related lectins strongly suggests that all belong to the same superfamily of lectins. A total of 12 amino acids of the C terminal are conserved among all mannose-binding lectins, which is essential for proper folding (Bourne et al., 1999). These proteins have three primarily conserved mannose-binding sites (Fig. 2(A)). Our results indicate that the mannose-binding sites of the lectins may not be conserved totally. OsJAC1 has the same mannose-binding sites as salT tested as a mannose-binding lectin (Zhang et al., 2000), so OsJAC1 may be a mannose-binding lectin.

Phylogenetic reconstruction with these nine protein sequences indicated that OsJAC1, together with BGAF, Ta-VER2 and 2 JA-induced proteins (Hv-JA1, Ta-JA1) belonged to one group, with Hfr-1 and Wci-1 belonging to another, Jacalin or salT by itself (Fig. 2(B)).

3.2. Expression of OsJAC1 by semi-quantitative RT-PCR

The expression of OsJAC1 was examined in various tissues by semi-quantitative RT-PCR. cDNA synthesis was performed using total RNA from roots, stems, leaves, young spikes and young seedlings as described above. The cDNA and gene-specific primers (OsJACF and OsJACR) were used to generate the OsJAC1 product, and tubulin was amplified in the same reaction to serve as an internal standard. OsJAC1 expression was detected in all tissues except for young roots. The expression was high in leaves and low in stems and spikes (Fig. 3(A)). When rice seedlings were treated with methyl jasmonate for 0, 6, 12 and 24 h, respectively, the expression levels of OsJAC1 increased, the highest level was for 6 h (Fig. 3(B)). Therefore, OsJAC1 may be involved in rice defence or that its products may display toxic properties.

3.3. Expression of the jacalin region

To test the activity of OsJAC1, the 3′-terminal sequence (438 bp) of the ORF added with the start codon (ATG) was inserted into a pGEX4T-1 vector and expressed in E. coli cells. The recombinant protein GST-OsJAC1 was visualized by Coomassie-blue stained PAGE (Fig. 4). The formation of recombinant protein was observed obviously after IPTG was added to the cell culture for 3 h (lane 3), the cell lysate supernatant contained the recombinant protein GST-OsJAC1 (lane 4). The strong band between 25 and 35 kDa in lane 3 was the GST band (26 kDa) of pGEX4T-1 vector with IPTG induction (lane 1), so the strong band was GST. The recombinant protein was purified successfully from the affinity matrix glutathione Sepharose-4B column (lane 5). The purified GST-OsJAC1 showed a relative molecular mass of 42 kDa.

3.4. Agglutination activity and carbohydrate-binding properties

ConA (Canavalia ensiformis, a mannose/glucose specific lectin), BSA (bovine serum albumin), and WS
(elution/washing buffer of the recombinant protein) were considered positive, negative and null controls, respectively. The concentration of ConA, BSA and GST-OsJAC1 was 1 mg/mL. ConA but not WS and BSA could agglutinate rabbit red blood cells. This result indicated that the tests were successful. Approximately 1.95 μg/ml GST-OsJAC1 could agglutinate rabbit erythrocytes, and approximately 0.98 μg/ml ConA (Fig. 5(A)). The carbohydrate-binding specificities of GST-OsJAC1 were determined by inhibition assays of the agglutination of rabbit red blood cells. The maximal concentration of GST-OsJAC1 inhibited by GlcNAc, Gal, Glc and Man was 1.30, 1.30, 2.60 and 10.42 μg/ml, respectively (Fig. 5(B)). The GlcNAc, Gal and Glc were 8, 8, and 4 times, respectively, less reactive than Man. So GST-OsJAC1 is best inhibited by Man. Thus, OsJAC1 is a mannose-binding jacalin-related lectin.

With respect to their molecular structure, sugar-binding specificity and temporal and spatial regulation, plant lectins are a widespread group of carbohydrate-binding proteins that show a marked heterogeneity. Over the past few years, the role of most lectins was associated with their binding to foreign glycans in recognition and/or defence-related phenomena, which has anti-nutritional/toxic effects on higher animals and insects (Vasconcelos and Oliveira, 2004). Most plant lectins target foreign glycans rather than endogenous plant carbohydrates (Peumans et al., 2000). However, recently, some evidence suggests that the location and regulation of the expression of these lectins are involved in specific endogenous protein–carbohydrate interactions within the cytoplasmic and/or nuclear compartment and hence can fulfil an important role in regulatory processes and signalling pathway in plant cell. This indicates that the role of these proteins goes beyond what has been proposed for the classical lectins (Van Damme et al., 2004). So plant lectins might also play a role in cellular regulation and signaling.

In summary, OsJAC1 expression is found in stems, leaves and young spikes but not roots of O. sativa. Methyl jasmonate can induce OsJAC1 expression. The recombinant O. sativa agglutinin was expressed in E. coli, and purified, showing significant agglutination activity towards rabbit red blood cells, which is well inhibited by Man, thus indicating that OsJAC1 is a mannose-binding jacalin-related lectin. The OsJAC1 and some proteins related to jacalin-related lectins from wheat and barley form a small protein family. This small protein family has evolved a disease-response domain in their N terminus from jacalin-related lectins (Wang and Ma, 2005). For instance, Wci-1 may be involved in systemic acquired resistance (Görlich et al., 1996), and Hv-JA1 can respond to Jasmonate (Lee et al., 1996). Ta-VER2 might be involved in vernalization process in wheat (Yong et al., 2003). These results may help reveal the functional role of these proteins to include the plant defense response. Therefore, OsJAC1 may play an important role in rice defence-related phenomena and/or cellular regulation and signaling.
Accession numbers

Accession numbers for the OsJAC1 sequences reported in this article are DQ243708 and ABB51090.

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References


