Novel Mannose-Binding Rice Lectin Composed of Some Isolectins and Its Relation to a Stress-Inducible salT Gene

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The novel mannose-binding rice lectin (MRL) purified by Sephadex G-50 or maltamyl Sepharose 4B affinity chromatography was not homogeneous, but the components were separated clearly by two dimensional polyacrylamide gel electrophoresis (1st; isoelectric focusing with Immobiline™, 2nd; SDS-PAGE). The major spots were located at pi 4.85 and 4.74, and minor spots at pi 4.66, 4.56, and 4.44; all spots were distributed at about MW 45,000. Other faint spots were sometimes detected just below the major spots. In the western blot analysis, all the spots reacted with the monoclonal antibodies specific to MRL, which bound to MRL and inhibited the lectin activity to agglutinate rabbit erythrocytes. The proteins of the spots at pi 4.85, 4.77, 4.66, and 4.56 had lectin activity. The major proteins at pi 4.85 and 4.77 also had the common amino acid sequence at N-terminus, TLVKIPWGGNGGSAQDISV, which is almost identical to salt and drought stress-inducible salT gene products in rice plants. High homology was also conserved in both the cDNA and the genomic clones encoding the MRL component at pi 4.85, which were selected with MRL-specific antibodies and an oligonucleotide designed from the partial amino acid sequence. All results suggest that MRL is composed of several isolectins, if not, related proteins having a common epitope and may belong to a family of stress-inducible proteins.

Key words: Isolectin — Mannose-binding lectin — Oryza sativa — salT gene — Stress-inducible proteins.

Many plant species contain carbohydrate-binding proteins, which are commonly referred to as lectins or agglutinins. Their physiological role in plants has not yet been elucidated. One of the possible roles is that lectins play a role in a plant’s defense mechanism owing to their binding specificity as does the immunoglobulin in the animal (Chrispeels and Raikhel 1991, Peuman and Van Damme 1995).

We found a novel rice lectin specific to mannose and glucose residues through studies on the interactions between the rice plant and its pathogens such as Xanthomonas campestris pv. oryzae, the pathogen of bacterial leaf blight of rice, and Magnaporthe grisea, rice blast fungus (Teraoka et al. 1990). Before our discovery, only seed lectins such as wheat germ agglutinin (WGA) and rice bran lectin (RBL) had been known in Poaceae. These seed lectins in Poaceae are similar in sugar specificity to N-acetylglucosamine and its β,1,4 linked oligomers, and their antisera cross-react with each other (Miller and Bowles 1983, Stinissen et al. 1983, Peuman et al. 1983, Tabary et al. 1987). On the other hand, this mannose-binding rice lectin (MRL) is distributed in all parts of the rice plant, and has a potential ability to agglutinate bacterial cells of X. campestris pv. oryzae and also spores and protoplasts of M. grisea. We have focused our attention on the hypothesis that the MRL may function as a plant antibody or a receptor in host-parasite recognition in the rice plant. Some evidence supporting our hypothesis obtained using a similar lectin specific to Man/Glc, Concanavalin A (Kanoh et al. 1988, Hamer et al. 1988, Xiao et al. 1994), has been reported. MRL was purified as a single band corresponding to MW 30,000 in SDS-PAGE, although a faint band was sometimes detected just below the major band. However, in the analysis by two dimensional PAGE, MRL was not homogenous; the major band was divided into several spots distributed in a broad range of pi 4.2 to 5.1 in the isoelectric focusing with Ampholyte in the first direction but at the same position in SDS-PAGE in the second direction. In this PAGE system, each major spot was not clearly separated by the isoelectric focusing with Ampholyte. Perhaps these spots are closely related at their isoelectric point. Although some of the spots may be contaminants, they all may be isolectins as reported for wheat germ agglutinin (WGA) (Allen et al. 1973, Rice and Ertsler 1975, Wright and Olafsdottir 1986, Smith and Raikhel 1989, Wright and Raikhel 1989), because of their ability to bind to Sephadex resin. One of the essential approaches to verify our hypothesis and clarify the possible role of MRL is to characterize the molecular properties of MRL and clone the gene(s). Here, we report that all components in the purified MRL preparation can be clearly separated into several spots by the modified PAGE technique, and that

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almost all of these spots may be isoelectins or closely related proteins in respect to their lectin activity, immunological specificity and partial amino acid sequence at the N-terminus. Furthermore, we will discuss the candidate of the MRL gene and its possible role on the basis of the homology search with known genes.

Materials and Methods

Plant material—MRL was purified from Oryza sativa L. cv. Aichi-asahi. The seedlings were grown in pots for 4 to 6 weeks in a greenhouse and divided into several parts after being washed sufficiently with tap water. Each part was stored at −40°C before use. The shoot-base including the shoot apex (4 to 5 cm in length from the bottom), which contains MRL with high specific activity, was mainly used.

For Northern blot analysis, the seedlings were grown hydroponically in half-strength Hoagland’s solution (Hoagland and Arnon 1938) at 26°C for 2 to 3 weeks in a greenhouse.

Purification of the mannos-binding rice lectin—The frozen sample was homogenized with a blender in 0.05 M HCl containing 5 mM β-mercaptoethanol and 0.2% (w/v) Polyclar AT. The homogenate was filtered through a double layer of cheesecloth, centrifuged at 10,000 x g for 30 min and the supernatant was precipitated with 10 to 50% saturation of (NH₄)₂SO₄. After dissolving the precipitate with 20 mM Tris-HCl (pH 7.1) and dialyzing against the same buffer, the solution was applied to a Q-Sepharose column equilibrated previously with the buffer. MRL bound to the resin was eluted with the buffer containing 200 mM NaCl. After dialyzing the active fractions against phosphate buffered saline (PBS), affinity chromatography with Sephadex G-50 or maltamyl Sepharose 4B resin was performed to fractionate MRL. MRL was eluted with PBS containing 200 mM glucose as a single peak. The active fraction was dialyzed against phosphate buffer (PB) containing 0.02% (w/v) sodium azide, and was concentrated by using CENTRIPREP-10 (Amicon) and stored at −80°C.

Lectin activity—Lectin activity was assayed by observing the hemagglutination of 1% (w/v) trypsin-treated rabbit erythrocytes in a serial double dilution of the test solution. The activity is represented as titer, which is the reciprocal of the highest dilution showing hemagglutination (Seyver 1962). The sugar specificity was assayed by mixing a series of dilution of the tested sugars with the lectin preparation adjusted to titer 4 with 10 mM PBS.

Electrophoresis for analysis of the MRL—The two dimensional PAGE (O’Farrel 1975) was modified to improve the resolution of the isoelectric focusing in the first direction; Immobiline Solution of the isoelectric focusing in the first direction; Immobiline (pH gradient from 3.5 to 5.0 in 5% (w/v) acrylamide gel, Pharmacia) was used instead of Ampholyte. The MRL preparation was mainly used.

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basis of the data of partial amino acid sequence at the N-terminus of the MRLs as shown in Table 2) labeled with [γ-32P]ATP by the end-labeling method with T4 polynucleotide kinase (Takara). The λ phages amplified in E. coli Y1090 were transferred onto Hybond-N+ nylon membrane (Amersham) and hybridized with the labeled oligonucleotide probe at 37°C in 6 x SSPE, 1 x Denhardt’s solution supplemented with 0.05% sodium pyrophosphate and 100 μg ml⁻¹ salmon sperm DNA. The membrane was washed in 6 x standard saline citrate (SSC) at room temperature and subsequently in 6 x SSC with 0.1% SDS at 47°C. Positive plaques were subsequently screened by using MRL specific monoclonal antibody No. 228. The finally selected positive plaques were amplified in E. coli Y1090 for isolating DNA, and their inserts were subcloned into pBluescript SK(+) for DNA sequencing by an ABI PRISM 377 (Applied Biosystems) DNA sequencing system.

Genomic DNA clones of the MRL—Genomic DNA was extracted from rice seedlings by using cetyltrimethylammonium bromide according to the method of Murray et al. (1980). Genomic DNA clones of the MRL 4.85 were obtained by a PCR method with the primers (primer 1; 5'-TTTAAGGACCAGCAGGAGATG-3', primer 2; 5'-AGTAATTCTTTGACCACTGG-3') which were designed on the basis of the sequence data of the MRL 4.85 cDNA clone. PCR was performed for 30 cycles of 94°C for 30 s, 54°C for 1 min and 72°C for 2 min. PCR product was cloned into T-vector constructed from pBluescript SK(+) by the method of Marchuk et al. (1990), which was designated pMRL and sequenced by the previous procedure.

Southern blot analysis—Rice genomic DNA (20 μg) digested with PsiI or BamHI was separated on 1% agarose gel electrophoresis and transferred to Hybond-N+ nylon membrane (Amersham). The genomic clone of MRL4.85 (pMRL) labeled with [α-32P]dCTP by using a Ready-To-Go DNA Labeling Kit (Pharmacia) was used as a probe. Hybridization was carried out in 500 mM Church phosphate buffer (pH 7.2), 1 mM EDTA and 7% SDS at 65°C (Church and Gilbert 1984). Washing was performed in 40 mM Church phosphate buffer (pH 7.2), 1% SDS at 65°C, followed by washing in 0.2 x SSC and 0.1% SDS at 65°C.

Northern blot analysis—Total RNA was extracted using Trizol Reagent (GIBCO BRL) as described above. An aliquot of RNA (20 μg) was separated on 1% agarose/formaldehyde gel and transferred to Hybond-N+ nylon membrane (Amersham). The membranes were prehybridized at 50°C in 5 x SSC, 10 x Denhardt’s solution supplemented with 10 mM sodium phosphate (pH 6.5), 0.5% SDS, 50% formamide and 100 μg ml⁻¹ salmon sperm DNA. The MRL cDNA labeled with DIG DNA Labeling Kit (Boehringer-Mannheim) was used as a probe. The membranes were washed in 2 x SSC with 0.1% SDS at room temperature and successively in 0.2 x SSC with 0.1% SDS at 55°C. The signals were detected with a DIG-DNA Detection Kit (Boehringer-Mannheim) according to the manufacturer’s instructions.

Results

Extraction, purification and separation of the mannos-binding rice lectin (MRL)—MRL could not be extracted by buffers at pH 3.5 to 5.0 or by deionized water, but by buffers at the other pH, most efficiently by 0.05 M HCl. Interestingly, 0.15 M NaCl solution was also effective for the extraction either by homogenizing with the solution or by only immersing in the solution for several hours (data not shown). In the last purification step, the MRL was eluted as a single peak with 200 mM glucose in 10 mM PBS from both Sephadex G-50 and maltamyl Sepharose 4B as described previously (Teraoka et al. 1990). Finally the MRL was purified about 56,000 to 60,000-fold in specific activity. The purified MRL, however, was not homogenous, but all components in the preparation could be separated by the modified two dimensional PAGE system with Immobiline gel in the first direction (Fig. 1A). The major spots were located at pI 4.85 and 4.74, the minor spots at pI 4.66, 4.56, and 4.44, whereas all spots were distributed at about MW 45,000. Other faint spots were sometimes detected just below the major spots corresponding to about MW 40,000. In some cases, regardless of the protein concentration applied, only the major spots at pI 4.85, 4.74,
and 4.66 corresponding to MW 45,000 were detected (data not shown). We tentatively refer to the major component at pI 4.85 as MRL 4.85, and the major one at pI 4.74 as MRL 4.74 hereafter. The major components, MRL 4.85 and MRL 4.74, could be isolated by using S-Sepharose ion exchange chromatography equilibrated with 20 mM acetate buffer (pH 4.0) and eluted with NaCl gradient in the buffer, although the resolution was inferior to that of the Immobiline PAGE system. When the MRL preparation was previously reduced with 5% (w/v) mercaptoethanol and 8 M urea by boiling for 5 min, all components shifted down to MW 15,000 without any change in the isoelectric point except for a new spot that appeared at pI 5.02 (Fig. 1C).

**Immunoblot analysis of the MRL**—Several hybrido-

### Table 1 Sugar specificity of MRL4.85, MRLs in preparation and Rice Bran Lectin (RBL)

<table>
<thead>
<tr>
<th>Tested sugar</th>
<th>MIC (mM)</th>
<th>Mannose-binding Rice Lectin</th>
<th>RBL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MRL4.85</td>
<td>Prepration</td>
</tr>
<tr>
<td>D-Mannose</td>
<td>2.5</td>
<td>6.25</td>
<td>&gt;100</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>5.0</td>
<td>18.8</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Methyl-D-mannose</td>
<td>3.13</td>
<td>2.5</td>
<td>&gt;200</td>
</tr>
<tr>
<td>Methyl-D-glucose</td>
<td>5.0</td>
<td>12.5</td>
<td>&gt;200</td>
</tr>
<tr>
<td>D-Glucosamine</td>
<td>12.5</td>
<td>25.0</td>
<td>&gt;100</td>
</tr>
<tr>
<td>N-Acetyl-D-glucosamine</td>
<td>5.0</td>
<td>12.5</td>
<td>6.25</td>
</tr>
<tr>
<td>D-Fructose</td>
<td>20.0</td>
<td>20.0</td>
<td>&gt;100</td>
</tr>
<tr>
<td>2-Deoxy-D-glucosamine</td>
<td>NT</td>
<td>18.8</td>
<td>NT</td>
</tr>
<tr>
<td>Maltose</td>
<td>6.25</td>
<td>6.25</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Trehalose</td>
<td>3.13</td>
<td>6.25</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Laminaribiose</td>
<td>5.0</td>
<td>5.0</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Isomaltose</td>
<td>10.0</td>
<td>12.5</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Sucrose</td>
<td>40.0</td>
<td>40.0</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Gentiobiose</td>
<td>50.0</td>
<td>50.0</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Maltiotol</td>
<td>12.5</td>
<td>12.5</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Isomaltiotol</td>
<td>20.0</td>
<td>20.0</td>
<td>&gt;100</td>
</tr>
<tr>
<td>N,N'-Diacetyl-chitobiose</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>0.125</td>
</tr>
<tr>
<td>N,N',N'-Triacetyl-chitotriose</td>
<td>&gt;0.4</td>
<td>&gt;0.4</td>
<td>0.02</td>
</tr>
<tr>
<td>D-Galactose</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>D-Xylose</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>D-Fucose</td>
<td>NT</td>
<td>&gt;100</td>
<td>NT</td>
</tr>
<tr>
<td>D-Ribose</td>
<td>NT</td>
<td>&gt;100</td>
<td>NT</td>
</tr>
<tr>
<td>D-Deoxyribose</td>
<td>NT</td>
<td>&gt;100</td>
<td>NT</td>
</tr>
<tr>
<td>D-Mannosamine</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>D-Galactosamine</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>N-Acetyl-D-mannosamine</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>N-Acetyl-D-galactosamine</td>
<td>NT</td>
<td>&gt;100</td>
<td>NT</td>
</tr>
<tr>
<td>L-Arabinose</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Cellobiose</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Lactose</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Melibiose</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Raffinose</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

* MIC: minimum inhibitory concentration at which the tested sugar completely inhibited hemagglutination. All lectin preparations tested were adjusted to titer 4 with PBS.
* The preparation purified by Sephadex G-50 affinity chromatography.
* The preparation isolated by S-Sepharose ion exchange chromatography (pH 4.0) following the affinity chromatography was used as MRL component corresponding to pI 4.85.
* RBL was purified by the method of Bloch and Burger (1974) and Tsuda (1979) after modification.
* NT: not tested.
ma cells that produce the monoclonal antibody specific to the MRL could be established by using the MRL purified by Sephadex G-50 as an antigen. All of the established clones had ability to bind to the MRL and also inhibit the lectin activity to agglutinate the rabbit erythrocytes. Perhaps these monoclonal antibodies recognize the sugar binding site or its vicinity in the MRL. In immunoblot analysis of the MRL purified using Sephadex G-50, all of the spots detected by CBB staining, shown in Fig. 1A, reacted with the monoclonal antibody specific to MRL (Fig. 1B). These results indicate that the components in the MRL purified using Sephadex G-50 may be derivatives of the MRL or, if not, related proteins which have a common epitope.

Lectin activity of the component protein in the MRL preparation—The proteins corresponding to pI 4.56, 4.66, 4.74, and 4.85 in the MRL preparation were recovered from the gel by the method of Casero et al. (1985). All proteins isolated had lectin activity to agglutinate trypsin-treated rabbit erythrocytes. Because the total titer was nearly proportional to its own strength in the CBB-staining, probably there is not a large difference in the agglutinating activity among the components. The sugar-binding specificity of the MRL 4.85, which was isolated by S-Sepharose ion exchange chromatography, was almost identical to that of the MRL preparation in the mixture (Table 1). This suggests that none of the components were contaminants but isolectins of the MRL and may have almost the same sugar-binding specificity at the mono- or oligosaccharide level.

Determination of N-terminal amino acid sequence of the MRL—Since the major components, MRL 4.85 and MRL 4.74, were always detected and quantitatively rich, their N-terminal amino acid sequences were determined after blotting the gel to Glassybond™ membrane. Thirty residues in MRL 4.85, and 20 residues in MRL 4.74 could be identified as shown in Table 2. Each component showed the same sequence in both reduced and native conditions. Of the amino acid sequences analyzed, 20 residues, TLVKIGPWGGNGGSAQDISV, were exactly identical (Table 2). A homology search using BLAST and Swiss Prot databases revealed that both MRL components were highly homologous to root-specific proteins in rice plants. Especially, the N-terminal amino acid sequence of MRL 4.85 was almost identical to that of the salT gene product that is induced by salt and drought stresses in the rice plant. There was only one difference in the 7th amino acid residue from the N-terminus, which is leucine in the salT but proline in MRL. This implies that MRL belongs to a family of stress-inducible proteins and also the expression might be regulated by some stress signals.

cDNA and genomic clones encoding the MRL—From the cDNA library constructed from healthy young rice seedlings, 17 clones were selected by a monoclonal antibody specific to the MRL and an oligonucleotide designed from data of the partial amino acid sequences. All the clones had absolutely the same DNA sequence as shown in Fig. 2. We could not isolate any clones with a different sequence from our library. The deduced amino acid sequence, partial amino acid sequence at the N-terminus, estimated isoelectric point and molecular weight were exactly the same as those of the reduced component of MRL 4.85. These clones were derived from at least one of the MRL gene(s). The cDNA sequence and its deduced amino acid sequence were almost identical to those of the salT gene and its product, not only in their partial sequences at 5' - or N-terminus but also in their overall sequences (Fig. 2); 10 nucleotides in cDNA and only 2 amino acid residues in deduced amino acid sequence were different between them. In the pMRL of the MRL 4.85 gene (Fig. 3),

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**Table 2** Amino acid sequence at N-terminus of the MRL(s) and their homologous sequence of proteins reported

<table>
<thead>
<tr>
<th>Protein</th>
<th>Amino acid sequence</th>
<th>Score</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRL4.85</td>
<td>TLVKIGPWGG NGGSAQDISV PPKKLLGVTI</td>
<td>30</td>
<td>Rice lectin specific to Glc/Man</td>
</tr>
<tr>
<td>MRL4.74</td>
<td>TLVKIGPWGG NGGSAQDISV</td>
<td>20/20</td>
<td></td>
</tr>
<tr>
<td>salT protein</td>
<td>TLVKIGPWGG NGGSAQDISV PPKKLLGVTI</td>
<td>29/30</td>
<td>Rice, root-specific, induced by</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>salt &amp; drought stress</td>
</tr>
<tr>
<td>GOS9 protein</td>
<td>LVKIGPWGG NGGGRVIDLSV LPSLKSVTI</td>
<td>19/29</td>
<td>Rice, root-specific</td>
</tr>
</tbody>
</table>

* Subscript figure represents the order of amino acid residue from N-terminus of the protein. Score means the number of the amino acid residues identical to those in MRL4.85 per total number of the amino acid residues compared. The boxed letters represent the different amino acid residue.

a Claes et al. (1990).

b Sylvia de Pater et al. (1992).
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-32 ATTACAAGGAAATTAAGCGACCACGAAGAGT
1 ATGACGCTGGTGAGATTGTGGTCGGAATATGGGAGCTAGCTGATCATCCATT

M h"LVKIGPWGGNGGSAQD!S|  20

1 ATGACGCTGGTGAGATTGTGGTCCGTGGGGCGGAAATGGAGGGTCAGCTCAGGACATCAGT

I  61 GTGCCACCCAAGAAGCTGTTAGGCGTGACAATCTACAGCTCAGATGCAATCAGATCCATT

|VPPKKLLGVTI|Y SSDAIRSI  40

121 GCCTTCAACTACATCGGTGTGGATGGACAGGAATATGCCATTGGTCCATGGGGTGCGGC

AFNYIGVDQEYAGPWGGG  60

181 GAAAGGCACCTACTCACAGATTAAACTGGGCTCCTCTGAGCAGATCAAGGAGATTTCTGGA

E G T S T E I K L G S S E Q I K E I S G  80

241 ACCCATGGCCCAGTCTATGATCTGGCTGACATTGTCACCTATCTTAAGATTGTGACAAGT

THGPVYDLADIVTYLKVITS 100

301 GCTAATAATACATGAGCTTGGATGATCCAAATGGGAAGTTACGACTTCCACTGCAA

ANNTYEAGVPNGKEFSIPLQ  120

361 GACTCTGGCCATTGCTGTTGGATTCTTTGGAAGGTCTGGAACGCTTATCGACGCAATTGGC

DSGHVVGFFGRSGTLIDAIG  140

421 ATCTAGTCCACCCTGTGATTTCCAGTTCAGTTGAAGATTACTACCTACTACCATATCTACG

IYVHP* 145

481 AAATAATGTTTCACTGTTGTGTTGTGACTTGCAATCCTCCCACTTCCACTGCAA

541 TGCCCGGATCGTGAATGCAATACAAAATGGGCGATATATATGTTGTGCTCCAAAAAAA

estimated pi; 4.86, estimated MW; 15060

Fig. 2 Nucleotide sequence and the deduced amino acid sequence of the MRL 4.85 gene, determined from the isolated cDNA insert. The boxed amino acid residues were determined by sequencing the N-terminal amino acid of the MRL 4.85 protein. Putative N-glycosylation site is underlined. Stop codon is indicated by asterisk (*). Arrowheads denote the amino acid residues different from the salT gene product. The isoelectric point and the molecular weight were estimated from the deduced amino acid sequence. Numbers in the left and the right margin indicate the order of nucleotides and amino acid residues, respectively.

which was identified by PCR, the homology with the salT gene was also conserved. The pMRL had 2 introns which were deduced from the same cDNA sequence as salT gene had (Garcia et al. 1998). In genomic Southern analysis using the whole pMRL as a probe, only one band in the BamHI-digested rice genome and 2 bands in the PstI-digested genome were detected (Fig.4). Based on the restriction map of BamHI and PstI site in pMRL as shown in Fig.4B, the MRL4.85 gene may exist as one copy in the genome. However, when hybridized in lower stringency, some additional bands were detected in both lanes (data not shown), suggesting the possibility that some similar sequences or other genes of the MRL exist in the genome.

Expression of the MRL 4.85 gene—The MRL 4.85 gene was highly homologous to the salT gene in both cDNA and genomic structure, strongly suggesting that MRL belongs to the same gene family as the salT gene. When rice plants were grown in non-submerged conditions, we were able to constantly detect the lectin activity of MRL in all plant parts at all growth stages. This indicates that the MRL gene(s) is constitutive. However, even such growth condition seems to give some water stress to the rice
plant, because we found that the components except for MRL4.85 and MRL4.74 sometimes disappeared even when purified by the same procedure. In our preliminary experiment with the seedlings that grew tall and thin, in a submerged condition (half-strength Hoagland’s solution) the MRL 4.85 mRNA was scarcely induced. In this condition, when NaCl (0.15 M), abscisic acid (5 μM) or jasmonate (5 μM) was added to the Hoagland’s solution, MRL 4.85 mRNA was induced in both root and shoot in the same way as the salT gene was induced (Fig. 5).
Discussion

Characterization of the components in the MRL preparation—The components in the MRL preparation were separated by using the modified two dimensional PAGE system with Immobiline in the first direction instead of Ampholyte. These components were the same in molecular weight, about 45,000 in the native condition and about 15,000 in the denatured, but slightly different in isoelectric point. Still, all the components reacted to the monoclonal antibody specific to the MRL, and the major ones had lectin activity to agglutinate rabbit erythrocytes. These results strongly suggest that all the components in the MRL preparation were truly isolectins of the MRL and not contaminants. If not, the components may be derivatives of proteins having a common epitope, in other words having a similar chemical structure, because the major components, MRL4.85 and MRL4.74, had a common amino acid sequence at their N-terminus. Probably all of the components have almost the same sugar-binding specificity and activity level as shown in Table 1. Previously, we reported that the molecular weight of the MRL was estimated to be about 30,000 by SDS-PAGE in the native condition, and that the MRL could not be stained with PAS reagent (Teraoka et al. 1990). In this study, however, the molecular weight was estimated to be about 45,000, although some faint spots appeared at the location corresponding to about MW 40,000 and the components were sometimes stained with PAS reagent. The molecular weight was always shifted after the modified two dimensional PAGE even in the same preparation whose molecular weight was estimated to be about 30,000 by SDS-PAGE. This inconsistency might result from the self-aggregation of the MRL via the sugar chain bound to MRL or the hydrophobic interaction especially near the isoelectric point or both. Following observations support this speculation. First, in the fractionation of MRL4.85 and MRL 4.74 by using S-Sepharose ion exchange chromatography, these components were not recovered from the resin by a pH gradient at all. Secondly, the MRL precipitate salted out with (NH₄)₂SO₄ could not be completely dissolved in some buffers, even with 0.05 M HCl solution. Thirdly, we often could not determine the molecular weight of MRL by gel filtration chromatography, because MRL in the native condition could not be eluted even from hydrophilic resins. In successful cases, the molecular weight was estimated to far exceed 45,000. In the Immobiline gel, the MRL may be easily self-aggregated, because the components were concentrated at their isoelectric points. A sugar chain might take part in the self-aggregation, because a putative N-glycosylation site exists in the deduced amino acid sequence of the MRL 4.85 gene product, without any cysteine residues in it. The inconsistency in PAS staining may be due to the degree of the modification of sugar chain in the MRL as has been sug-
suggested in rice bran lectin (Wilkin and Raikhel 1989). The modification may occur after the translation and depend on the growth condition or the stage of rice plant or both as well as the MRL gene regulation.

*Is the MRL gene(s) identical to the salT gene?—*The cDNA and the genomic clones encoding the MRL 4.85 were highly homologous to the salT gene induced by salt and drought stresses in the rice plant in both nucleotide sequences and the deduced amino acid sequence. The estimated molecular weight and isoelectric point of the salT gene product were also similar to those of MRL 4.85. Likewise, the MRL 4.85 gene was scarcely induced by a submerged condition, but induced by the treatment with abscisic acid and jasmonate in the same way as the salT gene. Probably the MRL 4.85 gene belongs to the same gene family as the salT gene. Further survey of the regulatory region and northern blot analysis of MRL may enable us to clarify not only the relationship with the salT gene but also the MRL expression in disease-stress. We suppose that the salT gene product has not yet been isolated and characterized, because 0.15 M NaCl solution extracts most of the gene product from rice plant at the same time.

*What is the origin of the MRL components?—*In this study we found some candidate clones and identified only one gene encoding the MRL 4.85. Southern blot analysis revealed that the MRL 4.85 gene may exist as one copy in the rice genome. However, when hybridized in lower stringency, several additional bands were detected, suggesting that some other genes for MRL exist in it. There still remains a question whether the other components originated from different genes, or from post-translational modification such as glycosylation or phosphorylation, or from a combination of both. Anyway, examining the MRL expression in diverse rice cultivars having different genotypes, or screening for other genes with a similar sequence to the MRL 4.85 gene, as suggested by the study of WGA (Wright and Olafsdottir 1986, Smith and Raikhel 1989, Wright and Raikhel 1989), may give us a clearer answer.

*Possible roles of the MRL in the rice plant—*The fact that the MRL has potential ability to agglutinate *X. campestris* and spores and protoplasts of *M. grisea* allowed us to hypothesize that MRL is involved in a non-self-recognition system, playing an antibody-like function. Now, data supporting our hypothesis are being accumulated by using the antibody specific to the MRL and the similar lectin, Concanavalin A. The presumption that the MRL gene, at least the MRL 4.85 gene, belongs to a stress-inducible gene family also strongly supports our hypothesis.

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### References


Tabary, F., Fout, J. and Bourrillon, R. (1987) Isolation, molecular and


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