Eur. J. Biochem. **269**, 4335–4341 (2002) © FEBS 2002 doi:10.1046/j.1432-1033.2002.03127.x

The lectin from leaves of Japanese cycad, *Cycas revoluta* Thunb. (gymnosperm) is a member of the jacalin-related family

Fumio Yagi[1,2], Toshinobu Iwaya[1], Tomokazu Haraguchi[1] and Irwin J. Goldstein[2]

[1]*Department of Biochemical Science and Technology, Faculty of Agriculture, Kagoshima University, Kagoshima, Japan;*
[2]*Department of Biological Chemistry, Medical School, The University of Michigan, Ann Arbor, Michigan, USA*

A novel lectin was isolated from leaves of the Japanese cycad, *Cycas revoluta* Thunb. (gymnosperm), and its characteristics including amino acid composition, molecular mass, carbohydrate binding specificity and partial amino acid sequences were examined. The inhibition analysis of hemagglutinating activity with various sugars showed that the lectin has a carbohydrate-binding specificity similar to those of mannose recognizing, jacalin-related lectins. Partial amino acid sequences of the lysylendopeptic peptides shows that the lectin might have a repeating structure and belong to the jacalin-related lectin family.

Keywords: *Cycas revoluta* Thunb.; gymnosperm; Japanese cycad; leaf; lectin.

Lectins have been isolated from many angiospermous plants including legumes and monocots [1,2]. Many applications have been found for their use in biological and bimedical research [3]. It has been suggested that plant lectins be classified into seven different protein families [4]. Thus far, only two isolectins have been isolated from seeds of a gymnospermous plant, that of Brazilian cedar, belonging to the family of Coniferae [5]. Except for these seed lectins, no lectins of gymnosperm origin have been purified; nor has the relationship between angiosperms and gymnosperms been established.

Previously, Pettitt [6] reported hemagglutinating activity in the extracts from cycad pollen. Until now, no other reports have appeared on lectin activity in other parts of cycad plants.

This paper deals with the isolation and characterization of the lectin present in the leaves of *Cycas revoluta* Thunb., a gymnosperm, and based on an examination of several lysylendopeptidase-derived peptides, its relationship to jacalin-related lectins.

MATERIALS AND METHODS

Materials

Leaves of Japanese cycad were collected on the university campus in Kagoshima, Japan, and were lyophilized and powdered.

Correspondence to F. Yagi, Department of Biochemical Science and Technology, Faculty of Agriculture, Kagoshima University, 1-21-24 Korimoto, Kagoshima city, Kagoshima 890-0065 Japan. Fax/Tel.: +81 99 285 3441, E-mail: fyagi@chem.agri.kagoshima-u.ac.jp

Abbreviations: CCA, *Castanea crenata* agglutinin (Japanese chestnut lectin); Con A, concanavalin A; CRLL, lectin from leaves of *Cycas revoluta* Thunb.

(Received 22 April 2002, revised 10 July 2002, accepted 22 July 2002)

Toyopearl HW 55F was purchased from Tosoh Ltd. (Tokyo, Japan), and mannose-agarose was from Sigma.

Saccharides

Various monosaccharides, methyl mannodisaccharides and glycoproteins were from Sigma. Manno-trisaccharides (Manα 1–2Manα1–2Man and Manα 1–6Manα1–6Man), Manα 1–3(Manα1–6)Man-α-*O*-Me and asparagine-glycopeptides (Man_5-Asp, Man_6-Asp and Man_9-Asp) were from a previous study.

Kojibiose, nigerose and sophorose were gifts from K. Takeo, the late professor of Kyoto prefectural University.

Asialoglycoproteins were prepared by desialylation with 0.1 M H_2SO_4 at 80 °C.

Hemagglutination assay

Hemagglutination assays were conducted in microtiter plates, in a final volume of 70 μL NaCl/P_i pH 7.2. Each well contained 50 μL lectin solution and 20 μL of a 4% (v/v) suspension of trypsinized erythrocytes.

Agglutination was assessed after incubation for 1 h at room temperature, and hemagglutinating activity was expressed as titer, namely, the reciprocal of the highest dilution that gave a positive result. The specific hemagglutinating activity was defined as titer (per mg lectin).

Quantitation of protein and carbohydrate

Protein was quantified by the method of Lowry *et al.* [7] with BSA as standard, and carbohydrate was quantified by the phenol/sulfuric acid method of Dubois *et al.* [8] with D-mannose as standard.

Electrophoresis

PAGE was carried out by the method of Davis [9] at pH 8.9. SDS/PAGE was performed using a discontinuous

system, as described by Laemmli [10]. Protein on all gels were stained with a silver staining kit (Wako Pure Chemicals).

Determination of molecular mass

The molecular mass of the purified lectin was estimated by gel filtration on a column of Toyopearl HW 55F (2.5 × 140 cm), by SDS/PAGE, and by HPLC. HPLC was performed with a model 880 PU pump, a model 875 UV detector and a Fine Pak SIL AF-102 column (JASCO Tokyo, Japan). The column was eluted with 10 mM sodium phosphate buffer pH 6.8, containing 0.2 M sodium sulfate at a flow rate of 0.5 mL·min^{-1}, and the effluent was monitored by absorption at 280 nm.

Amino acid analysis and determination of N-terminal amino acid sequence

The purified lectin (50 μg) was hydrolyzed with constant-boiling HCl in a sealed, evacuated glass tube for 48 h at 110 °C. The hydrolysates were analyzed with an amino acid analyzer (model 835; Hitachi). Cysteine was analyzed as cysteic acid after performic acid oxidation according to the method of Hirs [11].

N-Terminal sequencing was performed with a model 492 protein sequencer fitted with a model 140C PTH analyzer (Applied Biosystems-Perkin Elmer).

Lysylendopeptidase digestion and peptide separation

Lectin (0.1 mg) was denatured by treatment with 8 M urea in 50 mM Tris/HCl buffer pH 8.0, and dialyzed against 50 mM Tris/HCl buffer pH 9.0. Achromobactor lysylendopeptidase (Wako Pure Chemicals) was added to the substrate solution (enzyme : substrate, 1 : 100, w/w) and digestion was carried out at 37 °C for 18 h. Peptides were separated on a column of YMC Cel C4 300S-5 (4.6 × 250 mm) with a first gradient of 0–15% acetonitorile in 0.1% trifluoroacetic acid for 40 min and a second gradient of 15–75% acetonitrile in 0.1% trifluoroacetic acid for 5 min at 0.8 mL·min^{-1}.

The absorption of the effluent was monitored at 205 nm.

Purification procedure

Hemagglutinating activity was measured throughout all the purification procedures with trypsinized rabbit erythrocytes.

A total of 41.0 g leaf powder was extracted with 400 mL NaCl/P_i at 4 °C, and the homogenate was filtered through two layers of gauze and centrifuged at 8000 ***g***. Ammonium sulfate was added to the supernatant solution to 55% saturation. The precipitate was collected by centrifugation and dissolved in 20 mL NaCl/P_i. The solution was centrifuged to remove insoluble material. The supernatant solution was loaded onto a column of mannose–agarose (1.4 × 3.5 cm) previously equilibrated with 50 mM Tris/HCl buffer pH 7.5. The column was washed with 120 ml of the same buffer, and the lectin eluted with 0.2 M D-mannose in the same buffer. The lectin solution was dialyzed against 10 mM NH_4HCO_3 and lyophilized.

Quantitative precipitation

The microprecipitation procedure of So and Goldstein [12] was performed with some modifications [13].

RESULTS

Purification of lectin from leaves of Japanese cycad

It was found that the crude extracts from the leaves of the Japanese cycad (*Cycas revolute* Thunb.) agglutinated trypsinized rabbit, but not human, erythrocytes. A lectin was isolated from these extracts by one-step affinity chromatography on a mannose–agarose matrix. From 41.0 g leaf powder, 0.15 mg of the purified protein was obtained with a titer of 240 000 per mg lectin, showing a purification of 1700-fold from the crude extracts.

The homogeneity of the lectin preparation was examined by SDS/PAGE and native PAGE (Figs 1 and 2). A single protein band was observed by SDS/PAGE, whereas native PAGE gave a few bands, showing the presence of isolectins (Fig. 2). From the results of HPLC (Fig. 3) and SDS/PAGE, the molecular mass of the lectin was estimated to be ≈ 33 kDa, indicating the lectin to be a monomer.

Amino acid composition and N-terminal amino acid sequence of the lectin from Japanese cycad lectin

The amino acid composition of lectin from Japanese cycad is shown in Table 1, and compared with those of concanavalin A (Con A) [14] and lectins of Araucalia [5] and the Japanese chestnut [15]. As commonly found in lectins, the content of hydroxy- and dicarboxylic amino acids are high. Additionally, the content of glycine also is very high in these lectins, differing from that of Con A. The phenol/H_2SO_4 assay was negative indicating that the lectin is probably not

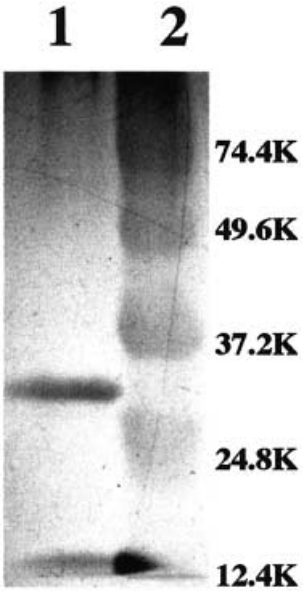

Fig. 1. SDS/PAGE (15% gel) of the final preparation. 1, CRLL without 10% 2-mercaptoethanol treatment; 2, molecular-mass marker (Wako Pure Chemicals).

Fig. 2. Native PAGE of affinity-purified CRLL.

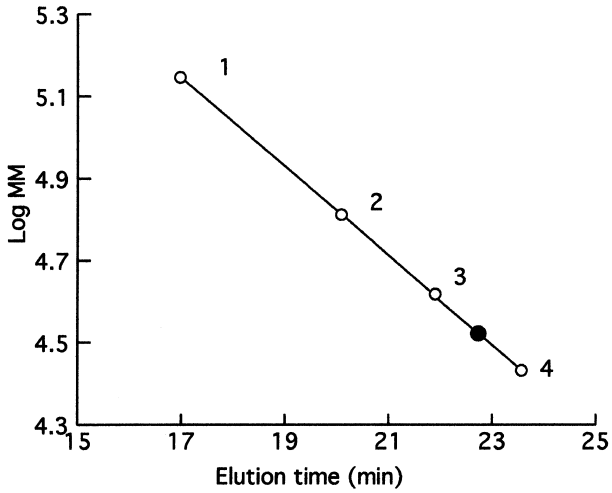

Fig. 3. Molecular mass estimation of CRLL on HPLC. ○, 1, alcohol dehydrogenase; 2, BSA; 3, ovalbumin; 4, luffin a. ●, Cycad leaf lectin.

a glycoprotein. The N-terminal amino acid of the lectin from leaves of *Cycas revoluta* Thunb. (CRLL) was not detected by the sequencer, suggesting that the lectin was blocked at its N-terminus.

Carbohydrate ligand inhibition of hemagglutination by mono- and oligo-saccharides and glycoconjugates

The hemagglutination inhibition by various saccharides is shown in Table 2. D-mannose was an eightfold more potent inhibitor than D-glucose, whereas Me-α-mannoside was equally inhibitory toward CRLL. *N*-Acetylglucosamine and D-glucosamine were also inhibitory but only one-fourth as potent as D-mannose.

Interestingly, Me-β-mannoside, Me-β-glucoside, and some saccharides with β-linkages were also inhibitory. Branched methyl mannotrioside was the most potent inhibitor among the oligosaccharides tested.

Table 3 shows the inhibition of hemagglutination by polysaccharides, glycopeptides and glycoproteins. The hemagglutination was strongly inhibited by yeast mannan of *Saccharomyces cerevisiae*, and N-linked high mannose glycopeptides and some glycoproteins with high mannose sugar chains. CRLL gave precipitin curves with yeast mannan and glycogen (Fig. 4), although glycogen was a weak inhibitor toward the lectin (Table 3).

Table 1. Amino acid compositions (mol/mol) of CRLL, *Araucalia* lectin and Con A.

Amino acid	CRLL	*Araucalia* II[a]	Con A[b]	CCA[c]
Asx	29.8	28.2	32	23
Thr	16.9	25.0	19	18
Ser	17.8	13.3	31	25
Glx	25.5	32.2	12	30
Pro	14.8	8.9	11	17
Gly	57.5	56.3	16	59
Ala	23.1	13.7	19	19
1/2Cys	0	19.3	0	1
Val	22.1	26.0	16	24
Met	1.1	0	2	6
Ile	19.2	19.0	15	24
Leu	17.3	11.9	18	21
Tyr	13.8	11.7	7	16
Phe	15.9	9.5	11	16
Lys	18.0	16.6	12	20
His	6.1	9.7	6	11
Arg	9.9	9.8	6	13
Trp	–	7.9	4	6

[a] Calculated from the data of Datta *et al.* [5]. [b] Cunningham *et al.* [14]. [c] Nomura *et al.* [15].

Comparison of sequence of cycad lectin peptides with three mannose recognizing jacalin-related lectins

Sequences of seven peptides obtained by lysylendopeptidase digestion are shown (Fig. 5). They could be assigned to the corresponding sequences of jacalin-related lectins [16–18] (Fig. 6). The N-terminal and C-terminal regions were highly conserved in jacalin-related lectins. At the regions close to N- and C-termini in heltuba, some residues are known to interact with mannose [19]. Peptides 12 and 15 were similar to the N-terminal region of one-domain lectin such as heltuba and calstegia, and peptides 19 and 20 were similar to the C-terminal region. It has not been determined where peptides 12 and 15 are located, near the N terminus or an inner region of the C-terminal domain. The position of peptides 19 and 20 are similarly not assigned.

DISCUSSION

Only two lectins have previously been isolated from a gymnosperm species, of the conifer family; no lectin has been isolated from other families of gymnosperm.

Pettitt [6] reported hemagglutinating activity present in cycad pollen. However, the principal of this hemagglutinating activity was not clarified. CRLL is apparently different from the pollen protein in its sugar-binding specificity and hemagglutinating activity. CRLL was inactive toward human erythrocytes. The molecular weight of CRLL was 33 kDa, close to those of the subunits reported for *Araucalia* lectin II and *Castanea crenata* agglutinin (CCA).

Table 2. Inhibition of hemagglutinating activity by mono- and oligosaccharides. Relative potency was to D-mannose. Galactose and sucrose were not inhibitory at 200 mM, *N*-acetyl D-galactosamine, D-xylose, D-lyxose and lactose were not inhibitory at 100 mM, and kojibiose (α1,2) and nigerose (α1,3) were not inhibitory at 50 mM.

Inhibitor	Minimum concentration (mM)	Relative inhibitory potency
Fructose	3.12	0.50
Glucose	12.5	0.125
Mannose	1.56	1.0
N-Acetyl D-glucosamine	6.25	0.25
D-Glucosamine	6.25	0.25
Me-α-D-glucoside	3.12	0.50
Me-β-D-glucoside	12.5	0.125
Me-α-D-Mannoside	1.56	1.0
Me-β-D-Mannoside	6.25	0.25
2-Deoxy-D-glucose	3.12	0.50
L-Fucose	100	0.015
Trehalose (α, α)	6.25	0.25
Sophorose (β1,2)	6.25	0.25
Laminaribiose (β1,3)	3.12	0.50
Laminaritiose	12.5	0.125
Laminaritetraose	12.5	0.125
Maltose (α1,4)	25.0	0.062
Cellobiose (β1,4)	50.0	0.031
Isomaltose (α1,6)	12.5	0.125
Gentiobiose (β1,6)	25.0	0.062
Manα1–2Man	2.23	0.70
Manβ1–2Man	1.12	1.39
Manα1–3Man	2.23	0.70
Manα1–3Man-α-*O*-Me	4.46	0.35
Manβ1–4Man	2.23	0.70
Manα1–4Man-α-*O*-Me	8.92	0.173
Manα1–6Man-α-*O*-Me	4.46	0.35
Manα1–6(Manα1–3)Man-α-*O*-Me	0.56	2.78
Manα1–2Manα1–2Man	8.92	0.173
Manα1–6Manα1–6Man	2.23	0.70

Table 3. Inhibition of hemagglutinating activity of cycad leaf lectin by polysaccharides, glycopeptides and glycoproteins. NI, No inhibition at the concentration given in parentheses.

Inhibitor	Minimum concentration
M_5glycopeptide	1.39 μM
M_6glycopeptide	13.9 μM
M_9glycopeptide	5.50 μM
Yeast mannan	15.6 $\mu g \cdot mL^{-1}$
Glycogen	500 $\mu g \cdot mL^{-1}$
Ovalbumin	31.3 $\mu g \cdot mL^{-1}$
Ovomucoid(Chicken)	125 $\mu g \cdot mL^{-1}$
Ovomucoid(Duck)	125 $\mu g \cdot mL^{-1}$
Ovomucoid(Quail)	7.81 $\mu g \cdot mL^{-1}$
Fetuin	125 $\mu g \cdot mL^{-1}$
Thyroglobulin	10.0 $\mu g \cdot mL^{-1}$
Asialothyroglobulin	5.0 $\mu g \cdot mL^{-1}$
Transferrin	NI (350 $\mu g \cdot mL^{-1}$)
Asialotransferrin	NI (350 $\mu g \cdot mL^{-1}$)

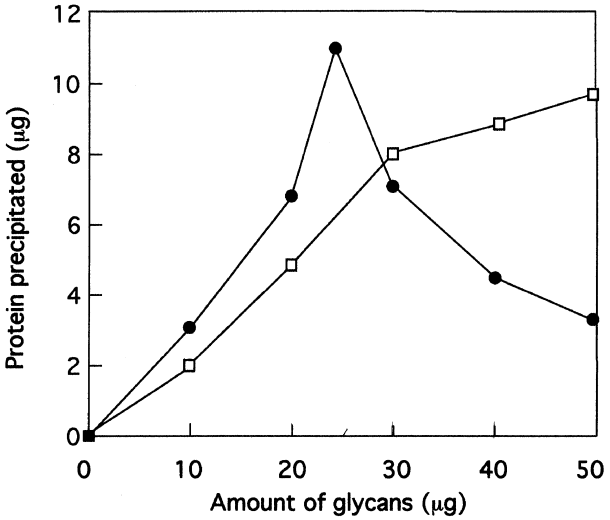

Fig. 4. Quantitative precipitation curves of CRLL with yeast mannan and glycogen. ●, yeast mannan and □, glycogen. Fifteen μg CRLL were mixed with various amount of glycans in a total of 120 μL NaCl/P_i. After 1 h incubation at 37 °C, the reaction mixtures were stored at 4 °C for 48 h. The precipitates formed were washed three times with 150 μL NaCl/P_i and dissolved in 0.05 M NaOH for protein assay.

The molecular mass of native CRLL was 33 kDa by HPLC, suggesting it to be a monomeric lectin. Conversely, the molecular masses of *Araucalia* lectins and CCA were 200 kDa. Amino acid composition of CRLL was very similar to those of *Araucalia* lectin II and CCA but different from those of legume lectins. Moreover, the sequences of some peptides of CRLL were very similar to mannose-specific, jacalin-related lectins. Of note, four peptides (12 and 15, 19 and 20, in Fig. 5) were similar to the sequences near the N terminus and the C terminus of jacalin-related lectins (Fig. 6). The common feature of jacalin-related lectins is a threefold symmetric β-prism fold based on crystallographic studies. The β-prism fold of lectins consist of three Greek key motifs. Greek keys 1 and 3 show the high conservation, and amino acid residues involved in the mannose-binding sites are present

```
 5  RYGPYGK
 7  SLTFHTNLTK
11  HGGPGGAATEIQFN--
12  ASVGYVVGGPWGGNGGDEWNDGTYQGIRK
15  EGPYGGVGGAPWDDGPQFGI--
19  IVGFYGRSGDYLDAIGVYAFTGV--
20  AEGFYGRVGTYLDFI--
```

Fig. 5. Partial sequences of lysylendopeptic peptides. The numbers of peptides are the elution order from a column of YMC Cel C4 300S-5 (4.6 × 250 mm).

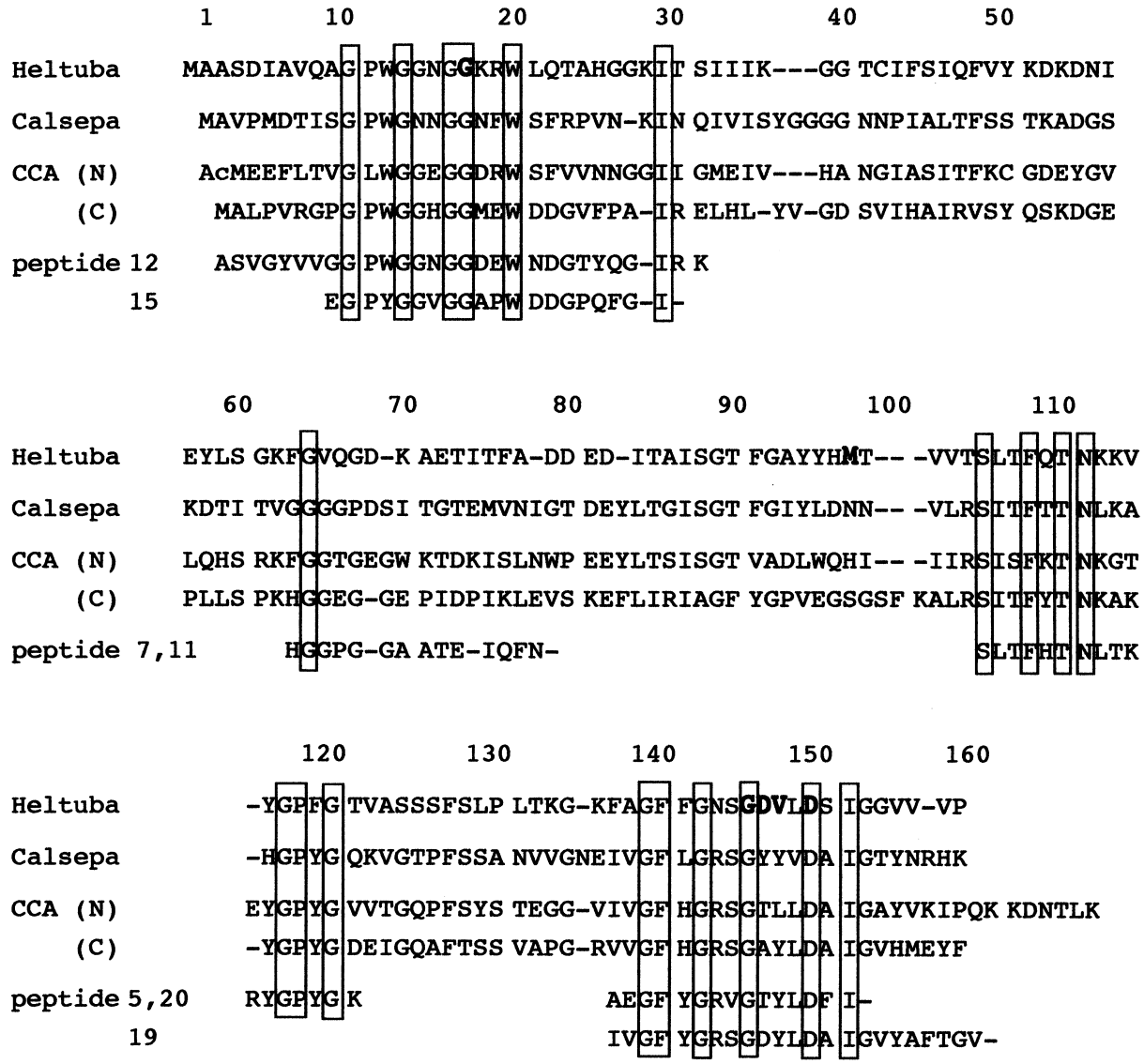

Fig. 6. Comparison of lysylendopeptic peptides with sequences of a few mannose-specific jacalin-related lectins. Bold letters are residues involved in mannose binding in heltuba [18]. Residues in frames are common in all the sequences. Sequences of calsepa and N-terminal and C-terminal domains of CCA are from Van Damme *et al.* [17] and Nomura *et al.* [16].

in the loop regions of Greek keys 1 and 3 (residues 1–26 and 136–158 in Greek key 1, and residues 82–135 in Greek key 3 in heltuba, Fig. 6).

CRLL has the sequences assigned to three Greek motifs. Peptides 12, 15, 19 and 20 correspond to the sequences in Greek key 1, peptide 7 in Greek key 2, and peptides 5 and 11 in Greek key 3. CRLL shows hemagglutination activity, though it is a monomeric protein. Therefore, CRLL might have two sugar binding sites. It seems likely that CRLL is a jacalin-related lectin with two homologous domains similar to CCA.

The cDNA structure of CRLL is now being analyzed. The structure will be elucidated in the near future.

The carbohydrate-binding specificity and hemagglutinating activity of CRLL are somewhat different from mannose-specific jacalin-related lectins reported thus far. CRLL showed a very strong hemagglutinating activity, but its activity was 10-fold lower than that of the *Araucalia* lectin.

The monomeric structure of CRLL may be responsible for its relatively low specific hemagglutinating activity. Heltuba weakly agglutinates trypsinized human erythrocytes [18], whereas CRLL was active only toward trypsinized rabbit erythrocytes. Maltose as well as D-mannose was strongly inhibitory toward one-domain jacalin-related lectins such as heltuba and calsepa [20], whereas maltose was a weak inhibitor of CRLL. Of the glycobioses tested, laminaribiose and Manβ1–2Man were the most potent inhibitors. Thus, CRLL also has affinity toward saccharides with β-linkages. In this regard, it resembles the banana lectin (banlec) which also recognizes laminaribiose [21]. Further, precipitation curves of CRLL with yeast mannan and glycogen (Fig. 4) were similar to those of banlec [13]. However, CRLL did not recognize nigerose and kojibiose, differing from banlec.

Of the glycopeptides, M_5-glycopeptide shows potent inhibition compared with branched trimannoside. On the

other hand, quail ovomucoid is a potent inhibitor and its inhibition concentration is calculated to be 2.6 μM based on its molecular mass 30 kDa. Furthermore, 50% of its sugar chain has been reported to be trimannosyl core [22]. Accordingly, it seems likely that M_3-glycopeptide is also a potent inhibitor. However, further study is necessary to confirm the potency by a quantitative method other than hemagglutination inhibition.

Van Damme *et al.* [4] reported that plant lectins were classified into seven groups. Most plant lectins are found in species belonging to a limited member of families or genera. For example, legume lectins are found only in leguminosae and mannose-specific monocot lectins are found in monocots except for the Gramineae. On the other hand, some animal lectins such as galectins and C-type lectins are found in widespread species.

Galectins are found in many animals from human to sponges. However, recent studies in plant lectin biochemistry have shown that mannose-specific, jacalin-related lectins also are widespread. These lectins are present in *Calystegia*, *Helianthus* and monocots including banana [23], and genes of jacalin-related lectins are found in *Arabidopsis*. Recently, Japanese chestnut lectin [16] was reported as a two-domain lectin with similar structures, and a lectin from the seeds of *Parkia platycephala* was shown to consist of three tandemly arranged jacalin-related lectins [24]. Thus, mannose-recognizing, jacalin-related lectins are distributed in a wide variety of species. Furthermore, the present study demonstrates that the mannose-specific, jacalin-related lectins are distributed not only in the angiosperms but also in gymnosperms.

The wide distribution of jacalin-related lectin may be related to their physiological role. In rice, some proteins were reported to be induced with the *salT* gene [25]. One of them has been assumed to be mannose-specific, jacalin-related lectin [26,27]. In *Helianthus*, the lectins are found to be induced by salt stress and methyljasmonates [28].

Whether CRLL and CCA with two similar domains are also induced by stress or methyljasmonate is now under study.

REFERENCES

1. Etzler, M.E. (1986) Distribution and function of plant lectins. In *The Lectins* (Liener, I.E. & Sharon, N. & Goldstein, I.J., eds), pp. 371–435. Academic Press Inc., Orlando FL.
2. Strosberg, A.D., Buffard, D., Lauwereys, M. & Foriers, A. (1986) Legume lectins: a large family of homologous proteins. In *The Lectins* (Liener, I.E. & Sharon, N. & Goldstein, I.J., eds), pp. 249–264. Academic Press Inc., Orlando FL.
3. Lis, H. & Sharon, N. (1986) Application of lectins. In *The Lectins* (Liener, I.E. & Sharon, N. & Goldstein, I.J., eds), pp. 293–370. Academic Press Inc, Orlando FL.
4. Van Damme, E.M.J., Peumans, W.J., Barre, A. & 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**, 575–692.
5. Datta, P.K., Figueroa, M.O.D.C.R. & Lajolo, F.M. (1991) Purification and characterization of two major lectins from *Araucaria brasiliensis* syn. *Araucaria angustifolia* Seeds (Penh). *Plant Physiol.* **97**, 856–862.
6. Pettitt, J.M. (1977) Detection in primitive gymnospermsof proteins and glycoproteins of possible significance in reproduction. *Nature* **266**, 530–532.
7. Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J. (1951) Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193**, 265–275.
8. Dubois, M., Gilles, K.A., Hamilton, H.K., Rebers, P.A. & Smith, F. (1956) Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **28**, 350–356.
9. Davis, B.J. (1964) Disc electrophoresis II, methods and application to human serum proteins. *Ann. NY Acad. Sci.* **121**, 404–427.
10. Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685.
11. Hirs, C.H.W. (1956) The oxidation of ribonuclease with performic acid. *J. Biol. Chem.* **219**, 611–621.
12. So, L.L. & Goldstein, I.J. (1967) Protein–carbohydrate interaction VI. Application of the quantitative precipitin method to polysaccharide–concanavalin A interaction. *J. Biol. Chem.* **242**, 1617–1622.
13. Mo. H., Winter, H.C., Van Damme, E.J.M., Peumans, W.J., Misaki, A. & Goldstein, I.J. (2001) Carbohydrate binding properties of banana (*Musa acuminata*) lectin I. Novel recognition of internal α1–3 linked glucosyl residues. *Eur. J. Biochem.* **268**, 2609–2615.
14. Cunningham, B.A., Wang, J.L., Waxdal, M.J. & Edelman, G.M. (1975) The covalent and three-dimensional structure of concanavalin A. II. Amino acid sequence of cyanogen bromide fragment F3. *J. Biol. Chem.* **250**, 1503–1512.
15. Nomura, K., Ashida, H., Uemura, N., Kushibe, S., Ozaki, T. & Yoshida, M. (1998) Purification and characterization of a mannose/glucose-specific lectin from *Castanea crenata*. *Phytochemistry* **49**, 667–673.
16. Nomura, K., Nakamura, S., Fujitake, M. & Nakanishi, T. (2000) Complete amino acid sequence of Japanese chestnuts agglutinin. *Biochem. Biophys. Res. Comm.* **276**, 23–28.
17. Van Damme, E.J.M., Barre, A., Verhaert, P., Rouge, P. & Peumans, W.J. (1996) Molecular cloning of the mitogenic mannose/maltose-specific rhizome lectin from *Calystegia sepium*. *FEBS Lett.* **397**, 352–356.
18. Van Damme, E.M.J., Barre, A., Mazard, A.M., Verhaert, P., Horman, A., Debray, H., Rouge, P. & Peumans, W.J. (1999) Characterization and molecular cloning of the lectin from *Helianthus tuberosus*. *Eur. J. Biochem.* **259**, 135–142.
19. Bourne, Y., Zaboni, V., Barre, A., Peumans, W.J., Van Damme, E.M.J. & Rouge, P. (1999) *Helianthus tuberosus* lectin reveals a widespread scaffold for mannose-binding lectins. *Structure* **7**, 1473–1482.
20. Peumans, W.J., Winter, H.C., Bemer, V., Van Leuven, F., Goldstein, I.J., Truffa-Bachi, P. & Van Damme, E.J.M. (1997) Isolation of a novel plant lectin with an unusual specificity from *Calystegia sepium*. *Glycoconjugate J.* **14**, 259–265.
21. Goldstein, I.J., Winter, H.C., Mo. H., Misaki, A., Van Damme, E.J.M. & Peumans, W.J. (2001) Carbohydrate binding properties of banana (*Musa acuminata*) lectin II. Binding of laminaribiose oligosaccharides and β-glucans containing β-1,6-glucosyl end groups. *Eur. J. Biochem.* **268**, 2616–2619.
22. Hase, S., Sugimoto, T., Takemoto, H., Ikenaka, T. & Schmid, K. (1986) The structure of sugar chains of Japanese quail ovomucoid. The occurrence of oligosaccharides not expected from the classical biosynthetic pathway for N-glycans; A method for the assessment of the structure of glycans present in picomolar amount. *J. Biochem.* **99**, 1725–1733.
23. Peumans, W.J., Zhang, W., Barre, A., Houles Astoul, C., Balint-Kurti, P.J., Robia, P., Rouge, P., May, G.D., Van Leuven, F., Truffa-Bachi, P. & Van Damme, E.J.M. (2000) Fruit-specific lectins from banana and plantain. *Planta* **211**, 546–554.
24. Mann, K., Farias, C.M., Del Sol, F.G., Santos, C.F., Grangeiro, T.B., Nagano, C.S., Cavada, B.S. & Calvete, J.J. (2001) The amino acid sequence of the glucose/mannose-specific lectin isolated from

Parkia platycephala seeds reveals three tandemly arranged jacalin-related domains. *Eur. J. Biochem.* **268**, 4414–4422.

25. Claes, B., Dekeyser, R., Villarroel, R., Van Den Bulcke, M., Bauw, G., Van Montagu, M. & Caplan, A. (1990) Characterization of a rice gene showing organ-specific expression in response to salt stress and drought. *Plant Cell* **2**, 19–27.
26. Hirano, K., Teraoka, T., Yamanaka, H., Harashima, A., Kunisaki, A., Takahashi, H. & Hosokawa, D. (2000) Novel mannose-binding rice lectin composed of some isolectins and its relation to a stress-inducible salT gene. *Plant Cell Physiol.* **41**, 258–267.
27. Zhang, W., Peumans, W.J., Barre, A., Astoul, C.H., Robia, P., Rouge, P., Proost, P., May, G.D., Truffa-Bachi, P., Jalali, A.A. & Van Damme, E.J.M. (2000) Isolation and characterization of a jacalin-related mannose-binding lectin from salt-stressed rice (*Oryza sativa*). *Planta* **211**, 970–978.
28. Nagasawa, R., Yasokawa, D., Okumura, Y. & Nakajima, K. (2000) Cloning and sequence analysis of cDNA coding for a lectin from *Helianthus tuberosus* callus and its jasmonate-induced expression. *Biosci. Biotechnol. Biochem.* **64**, 1247–1254.