Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 304 (2003) 91–97
BBRC
www.elsevier.com/locate/ybbrc

A structural basis for the difference in specificity between the two jacalin-related lectins from mulberry (*Morus nigra*) bark☆

Pierre Rougé,[a,*] Willy J. Peumans,[b] Annick Barre,[a] and Els J.M. Van Damme[c]

[a] *Signaux et Messages Cellulaires chez les Végétaux, UMR-CNRS 5546, Pôle de Biotechnologie végétale, Chemin de Borde-Rouge, Castanet Tolosan 31326, France*
[b] *Department of Applied Plant Sciences, Katholieke Universiteit Leuven, Leuven B-3001, Belgium*
[c] *Department of Molecular Biotechnology, Ghent University, Coupure Links 653, Ghent B-9000, Belgium*

Received 10 March 2003

Abstract

The activity and specificity of a galactose-specific and a mannose-specific jacalin-related lectin from the bark of the black mulberry (*Morus nigra*) tree has been re-investigated using different experimental approaches. Both lectins definitely behave as polyspecific lectins recognizing galactose, mannose, and glucose even though MornigaG and MornigaM interact preferentially with galactose and mannose, respectively. The exceptionally extended size of the carbohydrate-binding site of both lectins apparently accounts for their polyspecific character. Parallel studies with other mannose-specific jacalin-related lectins confirmed that their exclusive specificity towards mannose/glucose relies on a reduced size of their carbohydrate-binding site.

Keywords: Black mulberry lectins; Jacalin-related lectins; Polyspecific lectins; Carbohydrate-binding site

Hitherto, seven different families of structurally and evolutionary related carbohydrate-binding proteins (also known as lectins, agglutinins, or hemagglutinins) have been identified in plants [24]. The first members of the family of the jacalin-related lectins were identified in seeds of the Osage orange (*Maclura pomifera*) [1] and jackfruit (*Artocarpus integrifolia*) [16]. Until 1996, jacalin-related lectins were considered a small homogeneous family of galactose/T-antigen-binding agglutinins occurring exclusively in a small subgroup of the plant family Moraceae. However, this idea had to be abandoned after the identification of mannose-specific lectins with a high sequence similarity to jacalin in the hedge bindweed (*Calystegia sepium*; Convolvulaceae) [19,23], Jerusalem artichoke (*Helianthus tuberosus*; Asteraceae) [25], jackfruit (*Artocarpus integrifolia*; Moraceae) [21], rice (*Oryza sativa*; Gramineae) [3,28], and banana (*Musa acuminata*; Musaceae) [4]. In addition several mannose-specific lectins have been identified that are built up of polypeptides consisting of two or more tandemly arrayed jacalin domains like the lectins of the Japanese chestnut (*Castanea crenata*; Fagaceae) [18] and *Parkia platycephala* (Fabaceae) [13], and the myrosinase-binding proteins of *Brassica napus* (Brassicaceae) [9]. Recently, a lectin from the gymnosperm Japanese cycad (*Cycas revoluta*) has been identified as a new member of the jacalin-related lectins [27].

On the basis of the available data the family of jacalin-related lectins is sub-divided into galactose- and mannose-specific lectins. The galactose- and mannose-specific jacalin-related lectins differ not only in their specifity but also in the molecular structure of the protomers and their sub-cellular location. Jacalin and its galactose-specific homologues are built up of 'cleaved' protomers consisting of a heavy (α) and a light (β) polypeptide chain and are located in storage protein vacuoles [20]. In contrast, the mannose-specific jacalin-related lectins consist of 'intact' protomers and are located in the cytoplasm [20]. Comparative structural and specificity studies done with jacalin and the mannose-binding homologues from jackfruit (artocarpin or

☆ *Abbreviations:* Heltuba, *Helianthus tuberosus* agglutinin; MornigaG, galactose-specific *Morus nigra* agglutinin; MornigaM, mannose-specific *Morus nigra* agglutinin.

* Corresponding author. Fax: +33-5-61-17-59-94.
E-mail address: Pierre.Rouge@ipbs.fr (P. Rougé).

0006-291X/03/$ - see front matter © 2003 Elsevier Science (USA). All rights reserved.
doi:10.1016/S0006-291X(03)00538-2

KM+), *H. tuberosus* (Heltuba) and *C. sepium* (Calsepa) revealed that the differences in specificity between both sub-families of jacalin-related lectins rely on the presence or absence of a cleavage of the protomer. In the absence of a proteolytic cleavage of the lectin polypeptide in the vicinity of the binding site, the mannose-specific jacalin-related lectins possess an extra loop, which makes the binding site inaccessible to galactose. When the protomers are cleaved into an α- and β-chain, this extra loop is opened, giving rise to a more extended site that preferentially binds galactose but also accommodates glucose, sialic, acid and *N*-acetylmuramic acid [2].

Biochemical and molecular analyses revealed that the bark of the black mulberry (*Morus nigra*) tree contains large quantities of a galactose-specific jacalin-related lectin (called MornigaG) and a mannose-specific homologue (called MornigaM) [26]. Since both lectins share a high sequence identity and, in addition, MornigaM is in terms of agglutinating activity 2–3 orders of magnitude more potent than any previously described mannose-specific jacalin-related lectin, the MornigaG/MornigaM combination offers a unique opportunity, indeed, to refine our insights into the impact of the cleavage of the protomers on the specificity and activity of the jacalin-related lectins.

Materials and methods

Chemicals. Mannose, galactose, glucose, methyl-α-mannopyranoside, methyl-α-galactopyranoside, and methyl-α-glucopyranoside were purchased from Sigma. Sensor chips (CM 5), HBS (10 mM Hepes, 150 mM NaCl, and 3.0 mM EDTA, containing 0.05% BIAcore surfactant P20, pH 7.4) and all the chemicals required to activate the carboxymethylated dextran and immobilize the glycoproteins (100 mM *N*-hydroxysuccinimide, 400 mM *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride, and 1 M ethanolamine hydrochloride adjusted to pH 8.5 with NaOH) were obtained from Pharmacia Biosensor AB (Uppsala, Sweden). Human serotransferrin was a gift from Dr. H. Debray (UMR-CNRS 8576, Villeneuve d'Ascq, France). Desialylation of human serotransferrin (5 mg) was performed in the presence of 0.1 M trifluoroacetic acid (1 ml) at 80 °C for 1 h. After lyophilization, asialo-serotransferrin was diluted in methanol and lyophilized. This procedure was repeated three times to eliminate the remaining trifluoroacetic acid. Arcelin-1 was isolated from seeds of *Phaseolus vulgaris* cv. RAZ2 [8]. Other glycoproteins were from Sigma.

Isolation of MornigaG and MornigaM. MornigaG and MornigaM were isolated from a crude extract of *M. nigra* bark by successive affinity chromatography on Gal–Sepharose 4B and Man–Sepharose 4B, respectively, as described previously [26]. To ensure complete purity both lectins were subjected to three successive rounds of affinity chromatography.

Affinity chromatography with purified MornigaG and MornigaM. Additional affinity chromatography experiments were done to check the binding of MornigaG and MornigaM on immobilized Gal– or Man–Sepharose 4B. Purified lectins (100 mg) dissolved in 50 ml of 1 M $(NH_4)_2SO_4$ were loaded on a column (2.5 cm × 10 cm; ~50 ml bed volume) of Gal–Sepharose 4B or Man–Sepharose 4B equilibrated with 1 M $(NH_4)_2SO_4$. The column was washed with 1 M $(NH_4)_2SO_4$ until the A_{280} fell below 0.01. The bound lectin was eluted with 0.1 M galactose or 0.1 M mannose in 1 M $(NH_4)_2SO_4$.

Surface plasmon resonance measurements. The specific interaction of MornigaG and MornigaM with arcelin-1, asialofetuin, and desialylated human serotransferrin was analyzed by surface plasmon resonance (SPR) using a biosensor BIAcore 1000 (Pharmacia Biosensor AB).

For immobilization on sensor chips CM 5, ribonuclease B, arcelin-1, fetuin, asialofetuin, or desialylated human serotransferrin was used at a concentration of 1 mg ml^{-1} in 5 mM sodium acetate buffer (pH 4.0). According to the change of the SPR response (expressed in resonance units or RU) as a result of the immobilization on the carboxymethylated dextran layer covering the sensor chip, an estimated surface concentration of 10 ng mm^{-2} of dextran was obtained for the immobilized proteins. Ribonuclease B contains exclusively glycan chains of the high-mannose type N-linked to an Asn residue [12]. Arcelin-1 contains mainly N-linked oligosaccharide chains of the high-mannose type [8] whereas fetuin possesses a mixture of N- and O-linked glycans containing sialylated terminal Gal residues [5,6]. The glycan moiety of human serotransferrin consists mainly of *N*-glycans of the *N*-acetyllactosaminic type [15].

Solutions of 25–100 mg ml^{-1} MornigaG and MornigaM in HBS (pH 7.4) were injected for 5 min onto the glycoprotein-bound surface of the sensor chip at a flow rate of 5 μl min^{-1}. The change of the SPR response was monitored at 25 °C for 9.30 min. The same glycoprotein sensor chip surface was used repeatedly after removing the remaining immobilized jacalin by washing with 10 mM HCl for 2 min.

Inhibition by monosaccharides was analyzed by injecting 5–25 mM solutions of sugars in HBS (pH 7.4) for 5 min at a flow rate of 5 μl min^{-1}. The change of the SPR response was monitored at 25 °C for 9.30 min. Inhibition is expressed as the percentage of lectin desorbed from the bound glycoprotein (as compared to measurements done in the absence of sugars). Results are means of triplicate experiments.

Docking of monosaccharides into the binding sites of MornigaG and MornigaM. The docking of methyl-galactopyranoside and mannose into the binding sites of MornigaG and MornigaM, respectively, was performed on a Silicon Graphics O2 R10000 workstation, using the programs InsightII, Homology, and Discover (Accelrys, San Diego CA, USA). The three-dimensional models of MornigaG and MornigaM have been built previously from the X-ray coordinates of jacalin [22] and Heltuba [2], respectively [26]. The lowest apparent binding energy (E_{bind} expressed in kcal mol^{-1}) compatible with the hydrogen bonds (considering van der Waals interactions and strong [2.5 Å < dist(D–A) < 3.1 Å and 120° < ang(D–H–A)] and weak [2.5 Å < dist(D–A) < 3.5 Å and 105° < ang(D–H–A) < 120°] hydrogen bonds; with D: donor, A: acceptor, and H: hydrogen) found in the jacalin/methyl-galactopyranoside [22] (RCSB PDB code 1JAC) and the Heltuba/mannose [2] (RCSB PDB code 1C3M) complexes was calculated with the cvff forcefield of Discover and used to anchor the pyranose ring of the sugars into the binding sites of MornigaG and MornigaM. Cartoons were drawn with Molscript [11] and rendered with Bobscript [7] and Raster3D [14].

Molecular surfaces and electrostatic potentials were calculated and displayed with GRASP using the parse3 parameters [17]. The solvent probe radius used for molecular surfaces was 1.4 Å and a standard 2.0 Å-Stern layer was used to exclude ions from the molecular surface [10]. The inner and outer dielectric constants applied to the protein and the solvent were, respectively, fixed at 4.0 and 80.0 and the calculations were performed keeping a salt concentration equivalent to 0.145 M.

Results

Specificity assays

Hapten inhibition assays with a series of common simple sugars indicated that the agglutinating activity of MornigaG was only inhibited by galactose and its

derivatives (Table 1). Neither glucose nor mannose inhibited the agglutination of the rabbit red blood cells. MornigaM was not inhibited by galactose but was very sensitive to methyl-α-mannopyranoside and to a lesser extent to mannose ($IC_{50} = 12.5$ mM) and glucose (Table 1). Though the results of these hapten inhibition assays confirmed that MornigaG and MornigaM resemble the previously described jackfruit lectins jacalin and KM+, respectively, for what concerns their overall specificity, they were also indicative of important differences in fine-specificity between the homologues from mulberry and jackfruit.

A detailed analysis of the reactivity of both lectins towards various glycoproteins using surface plasmon resonance measurements showed that MornigaM and MornigaG readily interacted with immobilized ribonuclease B and arcelin-1 (Fig. 1A) (containing mainly *N*-glycans of the high-mannose type). Both lectins also interacted similarly with asialofetuin (Fig. 1B) and desialylated human serotransferrin (Fig. 1C) (which both possess terminal galactose residues). The reactivity towards ribonuclease B, arcelin-1, and asialofetuin clearly indicates that MornigaG and MornigaM recognize mannose- as well as galactose-containing glycans. Inhibition experiments analyzed by surface plasmon resonance showed that 25 mM galactose inhibited the interaction of MornigaG with immobilized asialofetuin by 35% whereas mannose used at the same concentration had a weak inhibitory effect (7% inhibition) (Fig. 2A). The interaction of MornigaM with immobilized asialofetuin was equally inhibited by 25 mM mannose (16% inhibition) and 25 mM galactose (15% inhibition) (Fig. 2B). These results suggest that even though both lectins recognize galactose- and mannose-containing glycans MornigaG interacts more specifically with galactose whereas MornigaM interacts better with mannose.

Similar studies of the interaction of MornigaG and MornigaM with immobilized arcelin-1 yielded different results (Figs. 2C and D). MornigaG (11% inhibition) and MornigaM (0% inhibition) were weakly or not inhibited by 25 mM galactose whereas 25 mM mannose caused 63% and 65% inhibition of MornigaG and MornigaM, respectively. Methyl-α-mannopyranoside inhibited the interaction of both lectins for 92%, indicating that the methylated sugar is a more potent inhibitor. Glucose also caused 14% and 12% inhibition of the interaction between arcelin-1 and MornigaG and MornigaM, respectively. When methyl-α-D-glucopyranoside was used, the inhibition increased up to 73% and 72% for MornigaG and MornigaM, respectively, indicating that methylation strongly increases the inhibitory potency of glucose. The results of the inhibition experiments of the interaction with arcelin-1 suggest that MornigaG and MornigaM exhibit a similar affinity towards the high-mannose glycans of this glycoprotein. This observation is somewhat unexpected for MornigaG because this lectin exhibited a clear preference for galactose when the interaction of the lectin was studied with asialofetuin. Irrespective of these discrepancies there is no doubt that both *M. nigra* lectins specifically interact with mannose, glucose, and galactose and accordingly have to be considered as polyspecific lectins.

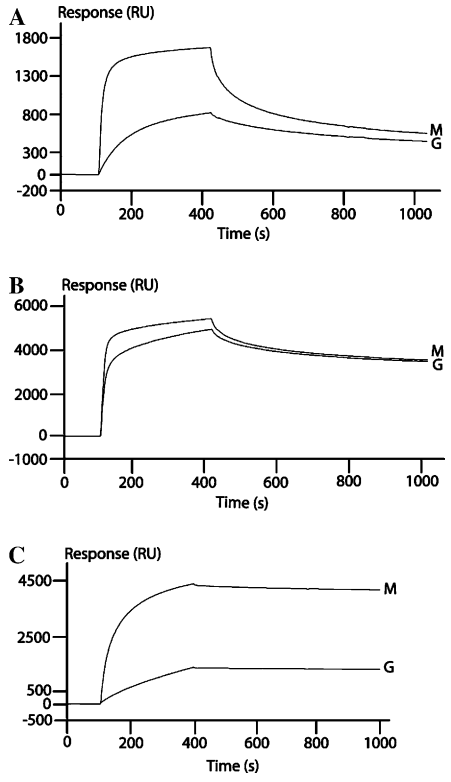

Fig. 1. Sensorgrams showing the interaction of MornigaM (M) and MornigaG (G) with immobilized arcelin-1 (A), asialofetuin (B), and desialylated human serotransferrin (C). Both lectins were used at a concentration of 100 μg ml^{-1} in HBS (pH 7.4). The amount of lectin bound to the immobilized glycoproteins is expressed in resonance units (RU). The upward and downward parts of the curves correspond to the association (circulating buffer containing the lectin) and the dissociation (circulating buffer) phases, respectively.

Table 1
Inhibition by simple sugars, sugar derivatives, and oligosaccharides of the agglutination activity of MornigaG and MornigaM towards trypsin-treated rabbit erythrocytes

Inhibitory sugar	IC_{50} (mM)	
	MornigaG	MornigaM
Galactose	25	>200
Methyl-α-galactopyranoside	25	>200
Glucose	>200	25
Methyl-α-glucopyranoside	>200	6.25
Mannose	>200	12.5
Methyl-α-mannopyranoside	>200	1.5
Sucrose	>200	>200
Maltose	>200	>200
Fructose	>200	>200

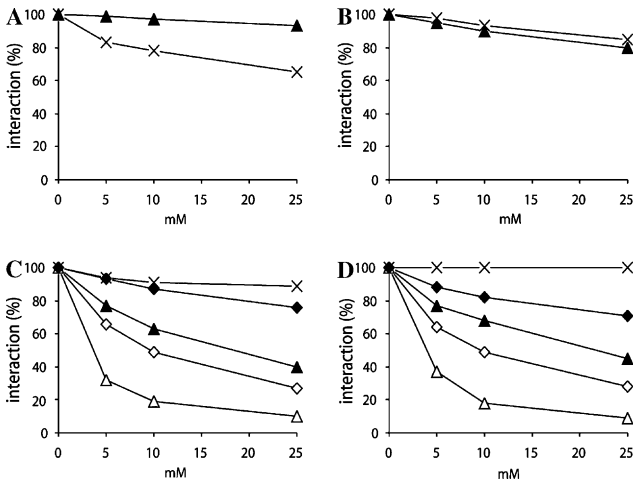

Fig. 2. SPR analysis of the specificity of MornigaG and MornigaM towards simple sugars and oligomannosides. Graphs A and B show the interaction of MornigaG (A) and MornigaM (B) with immobilized asialofetuin in the presence of galactose (×) and mannose (▲). Graphs C and D show the interaction of MornigaG (C) and MornigaM (D) with immobilized arcelin-1 in the presence of galactose (×), glucose (◆), mannose (▲), methyl-α-glucopyranoside (◇), and methyl-α-mannopyranoside (△). The sugars were added during the dissociation phase at concentrations of 5 mM up to 25 mM and results are expressed as the percentage of the lectins retained on immobilized asialofetuin or arcelin-1. Values are means of triplicate experiments.

To further investigate the polyspecificity of the *M. nigra* lectins, a series of simple affinity chromatography experiments on immobilized galactose and mannose was set up with purified MornigaG and MornigaM. MornigaG was quantitatively retained on both Gal–Sepharose 4B and Man–Sepharose 4B. In contrast, MornigaM was not retained on Gal–Sepharose 4B but bound exclusively to Man–Sepharose 4B. Neither MornigaG nor MornigaM was retained on unsubstituted Sepharose 4B. This different behavior confirms the differential specificity of MornigaG and MornigaM towards monosaccharides and the apparent more promiscuous specificity of MornigaG as compared to that of MornigaM.

Docking of monosaccharides in the binding sites of MornigaG and MornigaM

The structure of the binding sites of the *M. nigra* lectins was studied in more detail by docking experiments with simple sugars. As shown in Fig. 3A, the carbohydrate-binding site of MornigaG accommodates methyl-galactopyranoside through a network of eight hydrogen bonds connecting O3, O4, O5, and O6 of the sugar to residues Gly^{1}, Tyr^{122}, Trp^{123}, and Asp^{125}. Though this H-bonding scheme is similar to that occurring in jacalin there is a difference because a hydrogen bond between O6 and NH of Tyr^{122} of jacalin does not occur between O6 and NH of Tyr^{122} of MornigaG. Docking experiments also confirmed that mannose is reasonably well anchored in the carbohydrate-binding site of MornigaG by a network of six hydrogen bonds (Fig. 3C). Due to the equatorial position of O4 in mannose, two out of the four hydrogen bonds connecting the axial O4 of methyl-galactopyranoside to Gly^{1} and Asp^{125} can no longer occur in the mannose–MornigaG complex. Accordingly, O6 plays a key role in the interaction of mannose with MornigaG. Similar

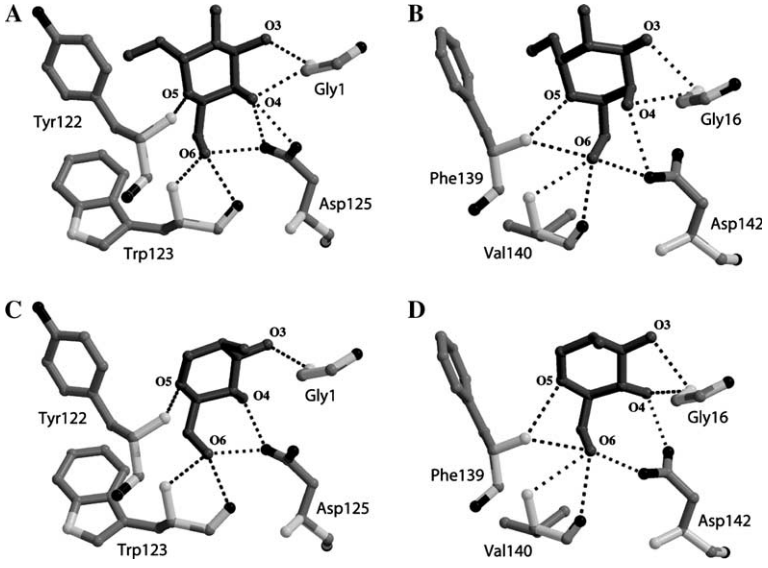

Fig. 3. Docking of methyl-α-galactopyranoside (A,B) and mannose (C,D) into the monosaccharide-binding sites of MornigaG (A,C) and MornigaM (B,D). Dashed lines correspond to the hydrogen bonds connecting the oxhydryls of the sugars (gray) to the amino acid residues of the binding sites (Gly^{1}, Tyr^{122}, Trp^{123}, and Asp^{125} in MornigaG; Gly^{16}, Phe^{139}, Val^{140}, and Asp^{142} in MornigaM). Amino acid residues and sugars are represented in ball-and-sticks.

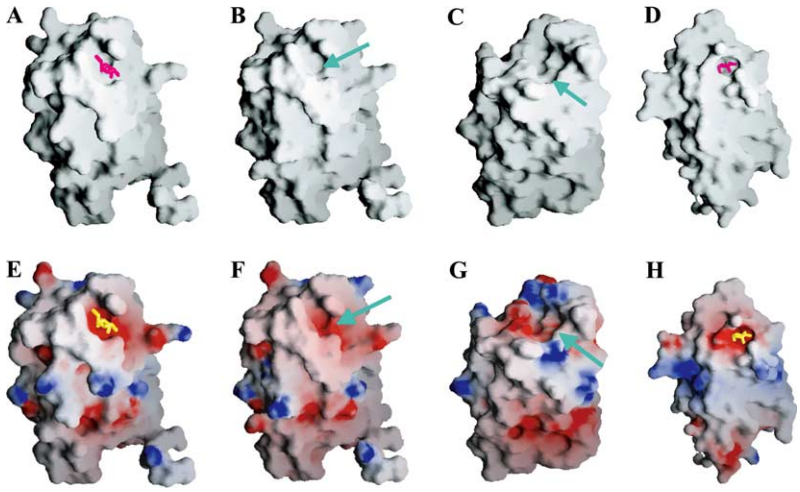

Fig. 4. Molecular surface and electrostatic potential maps of jacalin (A,E), MornigaG (B,F), MornigaM (C,G), and Heltuba (D,H) calculated and displayed with GRASP [17]. The monosaccharide-binding sites of jacalin (occupied by methyl-galactopyranoside), MornigaG, and MornigaM exhibit a more extended size than that occurring in Heltuba (occupied by mannose). Negative and positive potentials were colored gray and black and displayed at −5 and +5 levels (1 kT = 0.6 kcal), respectively. Neutral surfaces are white. Arrows indicate the location of the monosaccharide-binding site.

docking experiments indicated that the carbohydrate-binding site of MornigaM also can accommodate methyl-galactopyranoside and mannose (Figs. 3B and D).

The polyspecific character of the carbohydrate-binding sites of MornigaG and MornigaM is probably determined by their exceptionally extended size (Figs. 4B, F, C, and G). A comparison with Heltuba (Figs. 4D and H) clearly demonstrates, indeed, that the binding site of this typical mannose-binding jacalin-related lectin is smaller. Due to this smaller size Heltuba cannot interact with sugars possessing an axial O4 (e.g., galactose or methyl-galactopyranoside). A similarly extended carbohydrate-binding site occurs in jacalin (Figs. 4A and E), which also behaves as a polyspecific lectin [2]. All these carbohydrate-binding sites are similarly negatively charged because of the occurrence of acidic residues (Asp^{125} in MornigaG and jacalin, Asp^{142} in MornigaM, Asp^{136}, and Asp^{139} in Heltuba). However, due to a different orientation of O4 in galactose (axial) and mannose (equatorial) and a shift of the pyranose ring of mannose into the monosaccharide-binding site of MornigaG, the hydrogen-bonding schemes of galactose and mannose exhibit some differences. O4 of methyl-galactopyranoside is connected by four hydrogen bonds to the binding-site of MornigaG whereas O6, which is connected to the binding site by three distinct hydrogen bonds, becomes the key oxhydryl in the recognition of mannose by MornigaG. The network of hydrogen bonds anchoring mannose and methyl-galactopyranoside to residues forming the carbohydrate-binding site of MornigaM is very similar, which explains the reactivity of the lectin towards both sugars.

Discussion

Hitherto, the evolutionary and structural relationships between mannose-specific and galactose-specific jacalin-related lectins were not entirely clear because the models were based on a comparison of the sequences from unrelated species. It is very fortunate, therefore, that the cloning of MornigaG and MornigaM [26] allows one to corroborate the similarities/differences between mannose-specific and galactose-specific jacalin-related lectins in a single species and eventually refine the model of structure/specificity relationships of the family of the jacalin-related lectins in general and the Moraceae lectins in particular.

MornigaG clearly behaves as a polyspecific lectin recognizing galactose, mannose, and glucose. This polyspecific character apparently depends on the exceptionally extended size of the carbohydrate-binding site of MornigaG. In this respect, MornigaG resembles jacalin, which also possesses an extended binding site that can accommodate both galactose and mannose [2]. It is worth mentioning, however, that there is a difference between MornigaG and jacalin because the H-bonding scheme of methyl-galactopyranoside into the binding site comprises 8 and 9 hydrogen bonds, respectively. Structural studies revealed that the polyspecificity of jacalin is intimately linked to its complex biosynthesis. The conversion of pro-jacalin into the mature lectin protomer requires a proteolytic cleavage that takes place in the vicinity of the binding site. Due to this cleavage a loop is disrupted, which in turn results in the formation of an extended carbohydrate-binding site. Since MornigaG shares a very high sequence identity

with and undergoes the same post-translational modifications as jacalin, it is evident that the same reasoning can be followed to explain the polyspecific character of MornigaG. In contrast to jacalin and MornigaG, the protomers of the mannose-specific jacalin-related lectins such as Calsepa and Heltuba are not proteolytically cleaved. This implies that the loop corresponding to the 'cleaved' loop of jacalin remains intact. As a result of the presence of this loop the size of the carbohydrate-binding site of the mannose-specific jacalin-related lectins is reduced to such an extent that it accommodates exclusively mannose/glucose and accordingly the lectins do not exhibit a polyspecific character. Though this reasoning holds true for all previously characterized mannose-specific jacalin-related lectins the obvious polyspecific character of MornigaM indicates that the absence of a proteolytic cleavage of the lectin protomer does not necessarily prevent the formation of an extended binding site. Most probably the unusually extended carbohydrate-binding site of MornigaM results from local changes in the overall three-dimensional fold of its polypeptide chain. At present, it is not clear why MornigaM possesses a more extended sugar-binding site than its orthologues from other species. Possibly, the presence of the extended site is somehow related to the physiological role of MornigaM. Based on the spatial and temporal regulation of the expression it has been suggested that MornigaM is a typical vegetative storage protein with a possible defense-related role against insects and/or herbivorous animals [26]. The polyspecificity of MornigaM is in good agreement with the proposed defense-related role because the ability to recognize different sugars eventually enables the lectin to recognize and interact with a broad range of carbohydrate structures and accordingly widen the spectrum of possible target organisms [24]. Most probably, the broad range of target glycans also explains why MornigaM is 2–3 orders of magnitude more active in agglutination assays with animal and human erythrocytes.

Acknowledgments

We gratefully acknowledge the help of Dr. C. Houlès-Astoul and Dr. J.P. Borges for the BIAcore measurements.

References

[1] J.N. Bausch, R.D. Poretz, Purification and properties of the hemagglutinin from *Maclura pomifera* seeds, Biochemistry 16 (1977) 5790–5794.

[2] Y. Bourne, C. Houlès Astoul, V. Zamboni, W.J. Peumans, L. Menu-Bouaouiche, E.J.M. Van Damme, A. Barre, P. Rougé, Structural basis for the unusual carbohydrate-binding specificity of jacalin towards galactose and mannose, Biochem. J. 364 (2002) 173–179.

[3] B. Claes, R. Dekeyser, R. Villarroel, M. Van den Bulcke, G. Bauw, M. Van Montagu, A. Caplan, Characterization of a rice gene showing organ-specific expression in response to salt stress and drought, Plant Cell 2 (1990) 19–27.

[4] S.K. Clendennen, G.D. May, Differential gene expression in ripening banana fruit, Plant Physiol. 115 (1997) 463–469.

[5] D.A. Cummings, C.G. Hellerqvist, M. Harris-Brandts, S.W. Michnick, J.P. Carver, B. Bendiak, Structures of asparagine-linked oligosaccharides of the glycoprotein fetuin having sialic acid linked to *N*-acetylglucosamine, Biochemistry 28 (1989) 6500–6512.

[6] A.S.B. Edge, R.G. Spiro, Presence of an *O*-glycosidically linked hexasaccharide in fetuin, J. Biol. Chem. 262 (1987) 16135–16141.

[7] R.M. Esnouf, An extensively modified version of Molscript that includes greatly enhanced coloring capabilities, J. Mol. Graphics 15 (1997) 132–134.

[8] C. Fabre, H. Causse, L. Mourey, J. Koninkx, M. Rivière, H. Hendriks, G. Puzo, J.P. Samama, P. Rougé, Characterization and sugar-binding properties of arcelin-1, an insecticidal lectin-like protein isolated from kidney bean (*Phaseolus vulgaris* L. cv raz-2) seeds, Biochem. J. 329 (1998) 551–560.

[9] N. Geshi, A. Brandt, Two jasmonate-inducible myrosinase-binding proteins from *Brassica napus* L. seedlings with homology to jacalin, Planta 204 (1998) 295–304.

[10] M.K. Gilson, B.H. Honing, Calculation of electrostatic potential in an enzyme active site, Nature 330 (1987) 84–86.

[11] P.J. Kraulis, Molscript: a program to produce both detailed and schematic plots of protein structures, J. Appl. Cryst. 24 (1991) 946–950.

[12] C.J. Liang, K. Yamashita, A. Kobata, Structural study of the carbohydrate moiety of bovine pancreatic ribonuclease B, J. Biochem. 88 (1980) 51–58.

[13] K. Mann, C.M.S.A. Farias, F. Gallego Del Sol, C.F. Santos, T.B. Grangeiro, C.S. Nagano, B.S. Cavada, J.J. Calvete, 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 (2001) 4414–4422.

[14] E.A. Merritt, D.J. Bacon, Raster3D photorealistic molecular graphics, Methods Enzymol. 277 (1997) 505–524.

[15] J. Montreuil, Spatial conformation of glycans and glycoproteins, Biol. Cell 51 (1984) 115–132.

[16] R.A. Moreira, I.L. Ainouz, Lectins from seeds of jackfruit (*Artocarpus integrifolia* L.): isolation and purification of two isolectins from the albumin fraction, Biol. Plant. (Praha) 23 (1981) 186–192.

[17] A. Nicholls, K.A. Sharp, B. Honig, Protein folding and association: insights from the interfacial and thermodynamic properties of hydrocarbons, Proteins Struct. Funct. Genet. 11 (1991) 281–296.

[18] K. Nomura, S. Nakamura, M. Fujitake, T. Nakanishi, Complete amino acid sequence of Japanese chestnut agglutinin, Biochem. Biophys. Res. Commun. 276 (2000) 23–28.

[19] W.J. Peumans, H.C. Winter, V. Bemer, F. Van Leuven, I.J. Goldstein, P. Truffa-Bachi, E.J.M. Van Damme, Isolation of a novel plant lectin with an unusual specificity from *Calystegia sepium*, Glycoconjugate J. 14 (1997) 259–265.

[20] W.J. Peumans, B. Hause, E.J.M. Van Damme, The galactose-binding and mannose-binding jacalin-related lectins are located in different sub-cellular compartments, FEBS Lett. 477 (2000) 186–192.

[21] J.C. Rosa, P.S.L. De Oliveira, R. Garratt, L. Beltramini, K. Resing, M.-C. Roque-Barreira, L.J. Greene, KM+, a mannose-binding lectin from *Artocarpus integrifolia*: amino acid sequence, predicted tertiary structure, carbohydrate recognition, and analysis of the β-prism fold, Protein Sci. 8 (1999) 13–24.

[22] R. Sankaranarayanan, K. Sekar, R. Banerjee, V. Sharma, A. Surolia, M. Vijayan, A novel mode of carbohydrate recognition in

jacalin, a Moraceae plant lectin with a β-prism fold, Nat. Struct. Biol. 3 (1996) 596–603.
[23] E.J.M. Van Damme, A. Barre, P. Verhaert, P. Rougé, W.J. Peumans, Molecular cloning of the mitogenic mannose/maltose-specific rhizome lectin from *Calystegia sepium*, FEBS Lett. 397 (1996) 352–356.
[24] E.J.M. Van Damme, W.J. Peumans, A. Barre, P. Rougé, Plant lectins: a composite of several distinct families of structurally and evolutionary related proteins with diverse biological roles, Crit. Rev. Plant Sci. 17 (1998) 575–692.
[25] E.J.M. Van Damme, A. Barre, A.-M. Mazard, P. Verhaert, A. Horman, H. Debray, P. Rougé, W.J. Peumans, Characterization and molecular cloning of the lectin from *Helianthus tuberosus*, Eur. J. Biochem. 259 (1999) 135–142.
[26] E.J.M. Van Damme, J. Hu, A. Barre, C. Houlès-Astoul, P. Rougé, P. Proost, W.J. Peumans, Two distinct jacalin-related lectins with a different specificity and sub-cellular location are major vegetative storage proteins in the bark of the mulberry tree, Plant Physiol. 130 (2002) 757–769.
[27] F. Yagi, T. Iwaya, T. Haraguchi, I.J. Goldstein, The lectin from leaves of Japanese cycad, *Cycas revoluta* Thunb. (gymnosperm) is a member of the jacalin-related family, Eur. J. Biochem. 269 (2002) 4335–4341.
[28] W. Zhang, W.J. Peumans, A. Barre, C. Houles-Astoul, P. Rovira, P. Rougé, P. Proost, P. Truffa-Bachi, H.A. Jalali, E.J.M. Damme, Isolation and characterization of a jacalin-related mannose-binding lectin from salt-stressed rice (*Oryza sativa*) plants, Planta 210 (2000) 970–978.