Structure-function relationships of plant lectins that specifically recognize T and Tn antigens

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O-glycosylation is a widely distributed posttranslational modification responsible for the addition of GalNAc or GalNAc substituted with linear/branched sugar chains to serine or tyrosine residues of polypeptide chains. Some proteins like the mucins of vertebrates are extensively O-glycosylated and consist predominantly of carbohydrate. T antigens, which were originally designated as Thomsen-Friedenreich or TF antigens, are of particular interest in glycobiology since a high expression of the Tn antigen on the cell surface is diagnostic for cancer cells [1-3]. Due to the apparent loss of β–Galactosyl-transferase that normally converts the Tn antigen (GalNAcαO1-Thr/Ser) into the T antigen (Galβ1,3GalNAcαO1-Thr/Ser), the precursor Tn antigen accumulates in cancer cells (Fig. 1).

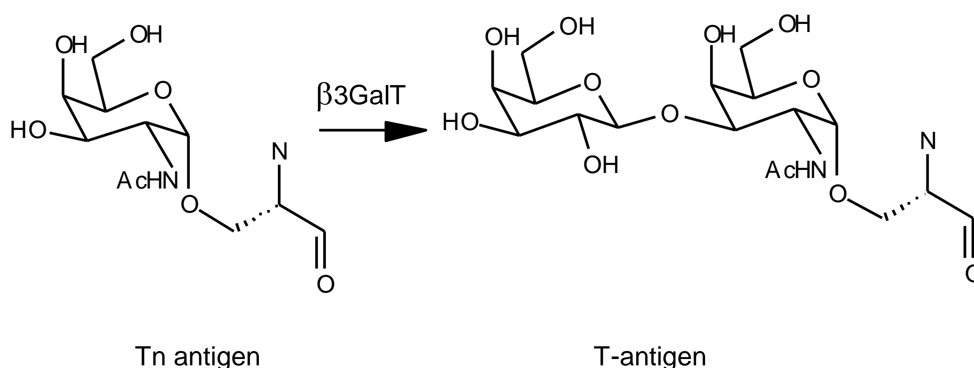

Figure 1. Conversion of Tn-antigen (GalNAcα1O-Ser) into T-antigen (Galβ1,3GalNAcα1O-Ser) by β–Galactosyl-transferase (β3GalT).

It is well established that the Tn antigen is a specific marker for human tumor cells, the presence of which is highly indicative for the carcinoma aggressiveness [4]. The Forssman

antigen (GalNAcα1,3GalNAcβ1,3Galα1,4Galβ1,4Glcβ1-1'ceramide) that shares a terminal GalNAc residue with Tn antigen is also expressed as a tumor-associated lipid antigen in different forms of cancers [5-9]. Hence proteins capable of specifically recognizing Tn and T antigens, e.g. monoclonal antibodies [10], are interesting tools for both the diagnosis and prognosis of carcinomas. In this respect, plant lectins that bind with a high specificity to Tn and T antigens constitute valuable tools provided they can discriminate between Tn/T antigens and other related *O*-glycans comprising exposed Gal or GalNAc residues. In the past, a whole list of plant lectins was described that interact with Tn and T antigens. However, only a few of them bind with a sufficiently high selectivity and affinity to be considered useful probes for the detection of Tn and T antigens. Moreover, even for these lectins the molecular basis for their selective binding to Tn/T antigens is still poorly understood. To further decipher the molecular basis for the Tn and T specificity we made a comprehensive analysis of the structural data provided by X-ray analysis of antigen-lectin complexes and complemented these data with novel insights generated by docking and modelling experiments.

I. OVERVIEW OF PLANT LECTINS DESCRIBED AS Tn AND T SPECIFIC

Numerous plant lectins are classified as Gal/GalNAc-specific lectins and accordingly interact to some extent with Tn or T antigens. However, lectins with an exclusive or preferential specificity for Tn and T antigens are rather scarcely distributed among higher plants. A survey of plant lectins that were described as T or Tn specific is presented in Table 1 [11,12]. Hitherto, T or Tn specific lectins have been found in the families Amaranthaceae, Fabaceae, Lamiaceae, Moraceae and Orchidaceae. The lectins themselves belong to 5 families of structurally and evolutionarily related proteins (amaranthins, legume lectins, jacalin-related lectins, type 2 ribosome-inactivating proteins and GNA-related lectins). Tn/T specific lectins are apparently fairly common in Fabaceae, Lamiaceae and Moraceae species. Interestingly, all Lamiaceae lectins identified thus far are genuine Tn-specific lectins. The same applies to the Amaranthaceae lectins. However, in the latter case lectins were only identified within a single genus.

Table 1: Plant lectins described as Tn/T antigen specific.

Lectin	Family	Structure	Specificity	Ref
Vicia villosa (VVLB4)		tetramer	Tn	14
Vicia graminea		tetramer	T cluster	25
Griffonia simplicifolia (GsLA4)		tetramer	Tn	15
Arachis hypogaea (PNA)		tetramer	T	16
Psophocarpus tetragonolobus (acidic WBAI)		dimer	T	17
Abrus precatorius		tetramer	T	26
Glechoma hederacea (Gleheda)		tetramer	Tn, T	31
Salvia sclarea		dimer	Tn	13
Salvia bogotensis		dimer	Tn	27
Salvia horminum		?	T	28
Molucella laevis (MLL)		?	Tn	29
Amaranthus caudatus (amaranthin)		dimer	T	18
Amaranthus leucocarpus		dimer	Tn, T	19
Laelia autumnalis		dimeric	T	20
Artocarpus integrifolia (jacalin)		tetramer	T	22
Maclura pomifera (MPA)		tetramer	T	21
Artocarpus lakoocha (ALA)		tetramer	Tn, T	23, 24
Morus nigra (Morniga-G)		tetramer	Tn, T	30

The clarity of the table can be improved by rearranging the different items
Classification according to plant family; mentioning the lectin family;

Table 1: Plant lectins described as Tn/T antigen specific.

Family/Species	Lectin family	Structure	Specificity	Ref
Amaranthaceae				
Amaranthus caudatus (amaranthin)	Amaranthin			
Amaranthus leucocarpus	Amaranthin			
Fabaceae				
Abrus precatorius	Type 2 RIP			
Arachis hypogaea (PNA)	Legume lectin			
Griffonia simplicifolia (GsLA4)	Legume lectin			
Psophocarpus tetragonolobus (acidic WBAI)	Legume lectin			
Vicia graminea	Legume lectin			
Vicia villosa (VVLB4)	Legume lectin			
Moraceae				
Artocarpus integrifolia (jacalin)	Jacalin-related			
Artocarpus lakoocha (ALA)	Jacalin-related			
Maclura pomifera (MPA)	Jacalin-related			
Morus nigra (Morniga-G)	Jacalin-related			
Lamiaceae				
Glechoma hederacea (Gleheda)	Legume lectin			
Molucella laevis (MLL)	Legume lectin			
Salvia sclarea	Legume lectin			
Salvia bogotensis	Legume lectin			
Salvia horminum	Legume lectin			
Orchidaceae				
Laelia autumnalis	GNA-related?			

II. RECENTLY CHARACTERIZED Tn AND T SPECIFIC PLANT LECTINS

Two promising novel plant lectins with a preferential affinity for Tn and T antigens were recently identified and characterized. One of them was isolated from leaves of the ground ivy (*Glechoma hederacea*, family Lamiaceae) and accordingly was called ***Gle**choma **hed**eracea* **a**gglutinin (or Gleheda) [30]. Cloning of Gleheda revealed that this typical Lamiaceae lectin belongs to the legume lectin family and possesses the typical three-dimensional organization known as the jelly roll scaffold, which is in fact a β-sandwich structure, similar to that found in legume lectins [30]. Moreover, a comparative analysis indicated that Gleheda and all other previously described Lamiaceae lectins represent a homogeneous subgroup of the legume lectin family. A second interesting novel Tn/T-specific lectin was purified from the bark of the black mulberry tree (*Morus nigra*, Moraceae) [31] and according to its origin was called Morniga-G (which stands for ***Morus nigra*** **G**al-specific **a**gglutinin) to distinguishe it from the conspecific mannose-specific homolog Morniga-M [32,33].

III. STRUCTURAL REQUIREMENTS FOR THE BINDING OF Tn ANTIGEN TO THE CARBOHYDRATE-BINDING SITE

At present, experimentally determined or modelled structural data are available for Tn-antigen binding lectins of the legume lectin and jacalin-related lectin families. The tetrameric *Vicia villosa* lectin VVLB4 was one of the first legume lectins to be described as a Tn-specific protein [35] and was also the very first for which the interaction with the Tn-antigen was solved by X-ray diffraction analysis of a lectin-Tn-antigen complex at 2.7-Å resolution (PDB code 1N47) [36]. Later, the structure of the basic lectin WBAI from winged bean (*Psophocarpus tetragonolobus*) in complex with Tn antigen (PDB code 2D3S) was X-ray solved at 2.35-Å resolution [37].

Structural analysis revealed that the Tn antigen anchors into the carbohydrate-binding site of VVLB4 by creating a network of eight hydrogen bonds between O3, O4, O6 and the *N*-acetyl group of the GalNAc residue and amino acid residues Asp85, Gly103, Asn129 and Asn214 (which form the conserved carbohydrate-binding pocket of the lectin) (Fig. 2A). Residue Tyr127 also plays a key role in the interaction because of a stacking interaction with the pyranose ring of GalNAc and an additional H-bond between the OH and the NH group of the Ser residue of Tn antigen. Hence, Tyr127 is considered a structural determinant of Tn-binding specificity of VVLB4 [36].

A very similar H-bonding scheme occurs in the carbohydrate-binding site of WBAI of *P. tetragonolobus* where O3, O4, O6 and the *N*-acetyl group of GalNAc form a network of seven H-bonds with His84, Asp87, Gly105 and Asp212 (Fig. 2B). Phe126 (homologous to Tyr127 of VVLB4) creates an additional stacking interaction with the pyranose ring of GalNAc. However, no direct H-bonding occurs between the Ser residue (of the Tn-antigen) and the lectin except for three H-bonds mediated by water molecules occupying the carbohydrate-binding pocket of the lectin (Fig. 2B). As a result, Tn antigen becomes specifically bound to the carbohydrate-binding site even though the aromatic residue Phe126 does due to its more pronounced hydrophobic character not play a key role in the binding. With the exception of water molecules, which introduce some specificity in the binding of the Tn antigen, the carbohydrate-binding site of WBAI interacts similarly with GalNAc and Gal residues as was shown from the WBAI-GalNAcα1-O-Me complex (PDB code 1DU1) [38]. Both GalNAc

residues exhibit the same orientation within the carbohydrate-binding pocket and are bound by a similar network of hydrogen bonds (Fig. 3).

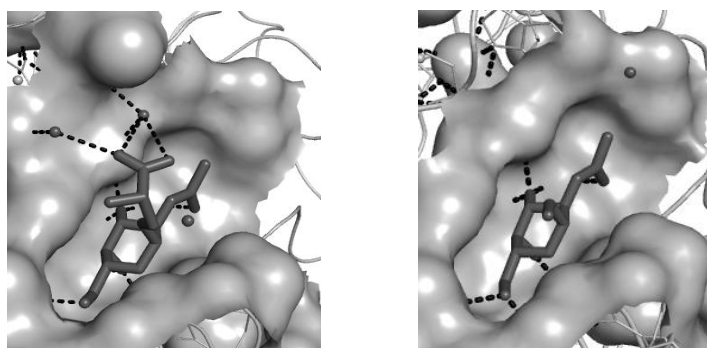

Figure 3. Docking of Tn antigen (left) and GalNAcα-O-Me (right) into the carbohydrate-binding pocket of WBAI. Note the different position of water molecules (grey balls) within the pocket. H-bonds are indicated by black dotted lines.

It seems more logic to discuss Gleheda immediately after the legume lectins (because it belongs to the same lectin family) and thereafter Morniga-G

The carbohydrate-binding site of the modelled Gleheda from *G. hederacea* accommodates the Tn antigen in a way very similar to that in the legume lectins VVLB4 and WBAI. As shown in Fig. 4, binding is established by a network of eleven H-bonds connecting O3, O4, O5, O6 and the N-acetyl group to residues Asp73, Gly93, Asn117, Thr201 and Asn202 (Fig. 4). Residue His115 creates an additional stacking interaction with the pyranose ring of GalNAc. Apparently, no binding occurs with the Ser residue (of the Tn-antigen) even though one cannot exclude the possible occurrence of water-mediated H-bonds like in the WBAI-Tn antigen complex.

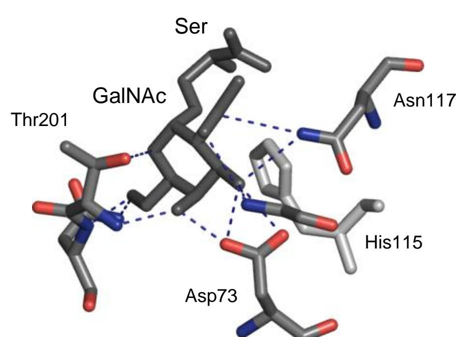

Figure 4. Docking of Tn antigen in the carbohydrate-binding site of Gleheda. Hydrogen bonds are indicated by black dotted lines. His115 that creates a stacking interaction with the pyranose ring of GalNAc is colored light grey.

Docking of the Tn antigen into the carbohydrate-binding site of the modelled Morniga-G from *M. nigra* (which belongs to the family of jacalin-related lectins) yielded an H-bonding scheme different from that found in the legume lectins VVLB4 and WBAI. As shown in Fig. 2C, H-bonds are formed between O3, O4, O5 and O6 of the GalNAc moiety and residues Gly1, Gly121, Tyr122, Trp123 and Asp125. Tyr78 interacts by a stacking with the pyranose ring of GalNAc and creates two additional H-bonds with the Ser residue (of the Tn-antigen) that is reminiscent of the binding of Tn antigen to VVLB4. No interaction was observed with the *N*-acetyl group of GalNAc.

The spatial arrangement of amino acid residues forming the carbohydrate-binding site that allows their hydrogen bonding to equatorial (O3, O4) oxygens and to the N-acetyl group of GalNAc, constitutes the molecular basis of the binding of Tn antigen to plant lectins. Two conserved Asp and Gly residues play a key role in this interaction. However, the specificity of the interaction depends on an additional residue (Tyr78 of Morniga-G, Tyr127 of VVLB4) which is close enough to the Ser residue (of the Tn-antigen) to create an additional H-bond. In other lectins (WBAI) water molecules present into the carbohydrate-binding pocket mediate H-bonds that specifically anchor the Ser residue (of the Tn-antigen) to the site. These additional H-bonds account for the enhanced affinity of these lectins for Tn antigen although the H-bonding scheme remains very similar to that observed for other simple sugars, e.g. Gal or GalNAc.

IV. STRUCTURAL REQUIREMENTS FOR THE BINDING OF T ANTIGEN TO THE CARBOHYDRATE-BINDING SITE

As shown in the overview given in Table 1, 4 or possibly even 5 different lectin families comprise T-antigen binding proteins. Hitherto, structural data are available for members of the jacalin-related lectins, the legume lectins and the amaranthins (and the type 2 ribosome-inactivating proteins???).

Due to its more bulky structure, the docking of T antigen disaccharide Galβ1,3GalNAc largely depends on the topography of the carbohydrate-binding pocket and surrounding area in T-specific lectins. Moreover, the affinity towards T antigen is often enhanced by its clustering at the surface of tumor cells [39] (this holds also true for the Tn-antigen!!!).

X-ray analysis of the corresponding lectin-T antigen-complexes demonstrated that the carbohydrate-binding site of the *Maclura pomifera* agglutinin (MPA) (PDB code 1JOT) [40] and jacalin from *Artocarpus integrifolia* (PDB code 1M26) [41], which both belong to the family Moraceae, accommodate the T antigen disaccharide in a similar way (Fig. 2D,E). The GalNAc residue binds to the carbohydrate-binding pocket through a network of nine H-bonds connecting O4, O5, O6 and the N-acetyl group to residues Gly1, Gly121, Tyr122, Trp123 and Asp125. An additional H-bond connects Gly1 to O3 engaged in the β1,3 O-glycosidic linkage between the Gal and GalNAc residues of T antigen. The Gal residue protrudes from the carbohydrate-binding pocket but remains connected to the lectin by a few water-mediated H-bonds. A very similar H-bonding network occurs in Morniga-G upon docking with the T antigen (Fig. 5). The GalNAc residue enters the carbohydrate-binding pocket whereas the Gal residue, which is not H-bonded to the binding site protrudes from the pocket like in the Tn antigen-jacalin and Tn antigen-MPA complexes (see Fig. 2D, E). However, water molecules associated to the carbohydrate-binding area that might create additional water-mediated H-bonds with the Gal residue were included in neither the modelling nor the docking experiments.

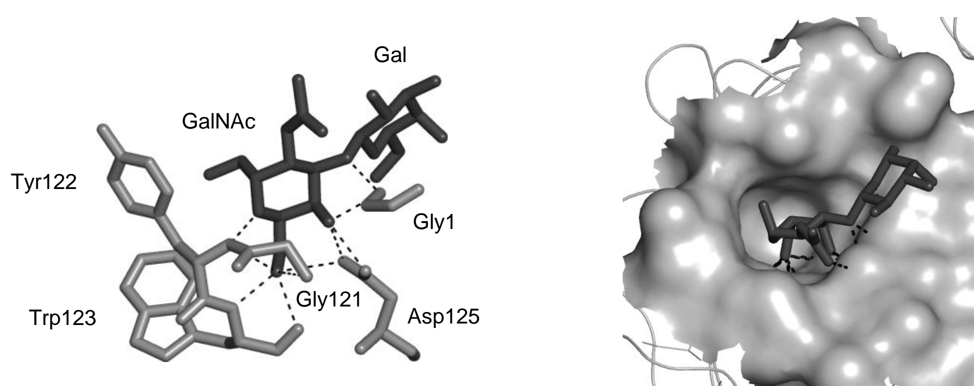

Figure 5. Docking of T antigen into the carbohydrate-binding pocket of Morniga-G (left) and surface analysis of the docking (right). T antigen is colored black and residues forming the carbohydrate-binding site are colored grey. H-bonds are indicated by black dotted lines.

Binding of the T antigen to PNA (PDB code 2TEP) [42], the peanut lectin, shows a rather different H-bonding scheme (Fig. 2F). The carbohydrate-binding pocket, which is shallower than that found in jacalin and the jacalin-related lectins MPA and Morniga-G, accommodates

the Gal residue through a network of eight H-bonds connecting O1, O3, O4 and O5 to residues Asp83, Gly104, Asn127 and Ser211. Tyr125 creates an additional stacking interaction with the pyranose ring of Gal. The GalNAc residue which protrudes from the carbohydrate-binding pocket is anchored to Ser211 and Gly212 residues by two H-bonds. Additional water-mediated H-bonds (attributed due to water molecules located in the vicinity of the carbohydrate-binding pocket) reinforce the interaction of both Gal and GalNAc with the lectin. This lectin-T antigen complex emphasizes the role of water molecules in the specific recognition of T antigen.

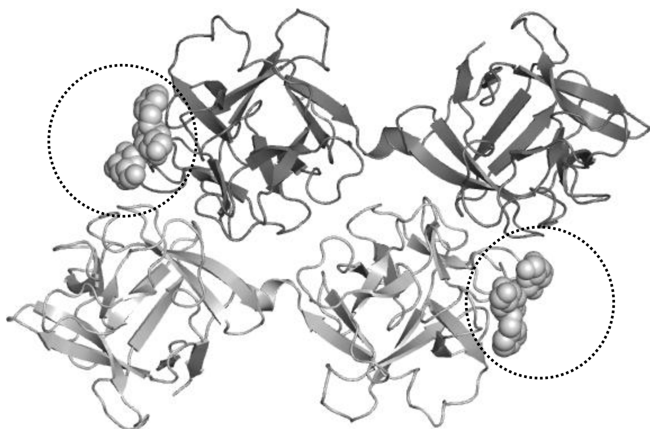

Figure 6. Three-dimensional structure of amaranthin in complex with benzyl-T antigen (grey CPK). Protomers A (deep grey) and B (light grey) are indicated. Residues of A and B chains participate in the carbohydrate-binding sites (circles) located at both ends of the dimer.

Amaranthin (PDB code 1JLX) [43] offers an unusual example of a T-specific homodimeric lectin with two identical carbohydrate-binding sites built up from amino acid residues located on the two protomers A and B. The lectin consists of two (identical) polypeptide chains A and B consisting of two in tandem arrayed domains with a β-trefoil fold connected by a short α-helical segment (Fig. 6). At both ends of the dimers, residues located on the A and B chains form a carbohydrate-binding site that specifically recognizes T antigen.

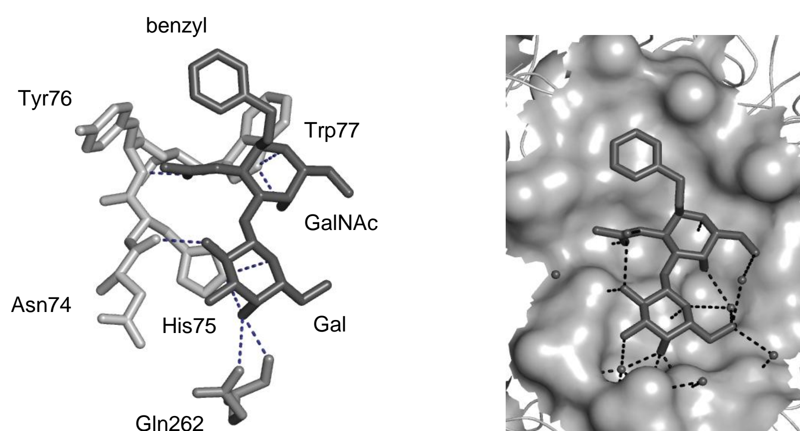

Figure 7. Docking of benzyl T antigen into the carbohydrate-binding site of amaranthin (left) and surface analysis of the docking (right). Benzyl T antigen is colored deep grey and residues forming the carbohydrate-binding site are colored light grey. Residue Gln262 belongs to chain B whereas other residues belong to chain A. H-bonds are indicated by dotted

lines. Water molecules at the surface of the carbohydrate-binding site are represented by grey balls.

The carbohydrate-binding site of amaranthin consist of residues Asn74, His75, Tyr76, Trp77 and Gln262 belonging to the A and B chain, respectively. They accommodate both Gal (5 H-bonds) and GalNAc (3 H-bonds) residues of benzyl T antigen through a network of eight H-bonds (Fig. 7). Additional water-mediated H-bonds connect both sugars to other residues located in the vicinity of the carbohydrate-binding site that reinforces the specific interaction of amaranthin with T antigen.

Abrin???

V. CONCLUDING REMARKS

Recognition of Tn antigen by plant lectins does not markedly differ from the recognition of GalNAc by Gal/GalNAc-specific lectins. Upon entering the carbohydrate-binding pocket of the Tn-specific lectin, the GalNAc moiety adopts an orientation similar to that found in other Gal/GalNAc-specific lectins. A similar network of H-bonds connects O3, O4, O6 and optionally the N-acetyl group of GalNAc to the different residues forming the carbohydrate-binding pocket. Two Asp and Gly residues (indicated by stars in Fig. 2) systematically act as key residues in the H-bonding. However, specificity (towards the Tn-antigen) is achieved either by additional H-bonds created with a close aromatic residue (VVLB4, Morniga-G) or by water-mediated H-bonds with residues located in the vicinity of the binding pocket (WBAI).
Depending on the lectins, the carbohydrate-binding pocket preferentially accommodates the GalNAc (jacalin, MPA, Morniga-G) or the Gal (PNA) residues upon binding of the T-antigen. The other sugar unit protrudes from the carbohydrate-binding pocket but remains usually connected to other residues located in the vicinity by water-mediated H-bonds. Obviously, the T-binding specificity depends of these additional water-mediated H-bonds that strongly reinforce the anchoring of T antigen into the binding site. Amaranthin differs from other T-specific lectins by a much more extended carbohydrate-binding site, which accommodates both sugar units of T antigen even though water-mediated H-bonds also play a key role in the T-binding specificity.
The binding of both Tn and T antigens to plant lectins stresses the importance of water molecules associated to the carbohydrate-binding site in enhancing the specificity of the ligand recognition.

References

1. Springer GF (1984) *Science* **24**, 1189-1260.
2. Springer GF (1997) *J. Mol. Med.* **75**, 594-602.
3. Itzkowitz SH, Bloom EJ, Kokal WA, Modin G, Hakomori SI, Kim YS (1990) *Cancer* **66**, 1960-1966.
4. Springer GF (1995) *Crit. Rev. Oncogen.* **6**, 57-85.
5. Hakomori SI (1984) *Am. J. Clin. Pathol.* **82**, 635-648.
6. Hakomori SI (1989) *Adv. Cancer Res.* **52**, 257-331.
7. Hakomori SI, Wang SM, Young WW (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3023-3027.
8. Taniguchi N, Yokosawa N, Narita M, Mitsuyama T, Makita A (1981) *J. Natl. Cancer Inst.* **67**, 577-583.
9. Yoda Y, Ishibashi T, Makita A (1980) *J. Biochem.* **88**, 1887-1890.

10. Hirohashi S, Clausen H, Yamada T, Shimossato Y, Hakomori SI (1985) *Proc. Natl. Acad. Sci. USA* **82**, 7039-7043.
11. Van Damme EJM, Peumans WJ, Pusztai A, Bardocz S (1997) In *Handbook of Plant Lectins: Properties and Biomedical Applications* (Eds. Van Damme EJM, Peumans WJ, Pusztai A, Bardocz) pp. 64-427, John Wiley & Sons, Chichester, UK.
12. Wu AM, Song SC, Tsai MS, Herp A (2001) *Adv. Exp. Med. Biol*. **491**, 551-85.
13. Piller V, Piller F, Cartron JP (1986) *J. Biol. Chem.* **261**, 14069-14075.
14. Puri K, Gopalakrishnan B, Surolia A (1992) *FEBS Lett.* **312**, 208-212.
15. Wu AM, Wu JH, Song SC, Kabat EA (1996) *FEBS Lett.* **398**, 183-186.
16. Lotan R, Skutelsky E, Danon D, Sharon N (1975) *J. Biol. Chem.* **250**, 8518-8523.
17. Patanjali SR, Sajjan U, Surolia A (1988) *Biochem. J.* **252**, 625-631.
18. Rinderle SJ, Goldstein IJ, Matta KL, Ratcliffe RM (1989) *J. Biol. Chem.* **264**, 16123-16131.
19. Zenteno E, Lescurain R, Montano LF, Vazquez L, Debray H, Montreuil J (1992) *Glycoconjugate J.* **9**, 204-208.
20. Zenteno R, Chavez R, Portugal D, Paez A, Lascurain R, Zenteno E (1995) *Phytochemistry* **40**, 651-655.
21. Sarkar M, Wu AM, Kabat EA (1981) *Arch. Biochem. Biophys.* **209**, 204-218.
22. Sastry MVK, Benerjee P, Patanjali SR, Swamy MJ, Swarnalatha GV, Surolia A (1986) *J. Biol. Chem.* **261**, 11726-11733.
23. Wu AM, Sugii S (1991) *Carbohydr. Res*. **213**, 127-143.
24. Singh T, Chatterjee U, Wu JH, Chatterjee BP, Wu AM (2005) *Glycobiology* **15**, 67-78.
25. Goldstein IJ, Poretz RD (1986) in *The lectins: Properties, Functions, and Applications in Biology and Medicine* (eds IE Liener, N. Sharon, IJ Goldstein) pp. 33-247, Academic Press, Orlando, USA.
26. Wu AM, Lin SR, Chin LK, Chow LP, Lin JY (1992) *J. Biol. Chem.* **267**, 19130-19139.
27. Vega N, Perez G (2006) *Phytochemistry* **67**, 347-355.
28. Bird GWG, Wingham J (1974) *Vox Sanguinis* **26**, 163-166.
29. Duk M, Mitra D, Lisowska E, Kabat EA, Sharon N, Lis H (1992) *Carbohydr. Res*. **236**, 245-258.
30. Wang W, Peumans WJ, Rougé P, Rossi C, Proost P, Chen J, Van Damme EJM (2003) *Plant J.* **33**, 293-304.
31. Van Damme EJM, Hause B, Hu J, Barre A, Rougé P, Proost P, Peumans WJ (2002) *Plant Physiol.* **130**, 757-769.
32. Singh T, Wu JH, Peumans WJ, Rougé P, Van Damme EJM, Alvarez RA, Blixt O, Wu AM (2006) *Biochem. J.* **393**, 331-341.
33. Singh T, Wu JH, Peumans WJ, Rougé P, Van Damme EJM, Wu AM (2007) *Mol. Immunol.* **44**, 451-462.
34. Sankaranarayanan R, Sekar K, Banerjee R, Sharma V, Surolia A, Vijayan M (1996) *Nat. Struct. Biol.* **3**, 596-603.
35. Tollefsen S, Kornfeld R (1983) *J. Biol. Chem.* **258**, 5165-5171.
36. Babino A, Tello D, Rojas A, Bay S, Osinaga E, Alzari PM (2003) *FEBS Lett.* **536**, 106-110.
37. Kulkarni KA, Sinha S, Katiyar S, Surolia A, Vijayan M, Suguna K (2005) *FEBS Lett.* **579**, 6775-6780.
38. Kulkarni KA, Katiyar S, Surolia A, Vijayan M, Suguna K (2006) *Acta Cryst.* **D62**, 1319-1324.
39. Wu AM (2004) *FEBS Lett.* **562**, 51-58.
40. Lee X, Thompson A, Zhiming Z, Ton-That H, Biesterfeldt J, Ogata C, Xu L, Johnston RAZ, Young NM (1998) *J. Biol. Chem.* **273**, 6312-6318.
41. Jeyaprakash AA, Rani PG, Reddy GB, Banumathi S, Betzel C, Surolia A, Vijayan M (2002) *J. Mol. Biol.* **321**, 637-645.
42. Ravishankar R, Ravindran M, Suguna K, Surolia A, Vijayan M (1997) *Curr. Sci.* **72**, 855-861.
43. Transue TR, Smith AK, Mo H, Goldstein IJ, Saper MA (1997) *Nat. Struct. Biol.* **4**, 779-783.