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Legume Lectins : A Promising Candidate for Confronting a Plethora of Biotic Stresses

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Abstract

Legume lectins are largest and best characterized families of plant lectins. These are homologous carbohydrate binding proteins that are found mainly in the seeds of legume plants. Despite their strong similarity on the level of their amino acid sequences and tertiary structures, their carbohydrate specificities and quaternary structures vary widely. In this review we will focus on the structural features of legume lectins and their complexes with carbohydrates. Legume lectins comprise a structurally related, Ca/Mn-dependent, widespread, abundant and well characterized lectin family when compared to the large number of lectins from other sources described in the literature. They have at least one non-catalytic domain that binds reversibly to specific monosaccharides or oligosaccharides. Legume lectins have diverse of activity such as antimicrobial, insecticidal activities, antitumor, immune-modulatory, and HIV-1 reverse transcriptase inhibitory, which may find applications in many therapeutic areas.

Introduction

Lectins are the carbohydrate binding proteins of non immune origin, possessing at least one non catalytic domain that binds reversibly and specifically to carbohydrates without altering their structure. Lectins recognize and bind to cell surface glycans exertingimmuno-modulatory effects. Lectins from different plant species often differ with respect to their molecular structure and specificity(Peumans and Van Damme, 1995). They are ubiquitous occurring in all forms of life including fungi and bacteria (Sharon, 2007).Most of the constitutively expressed lectins are synthesized with a signal peptide, and are sequestered in the vacuole or secreted to the extra- cellular space. In contrast, most of the inducible plant lectins reside in the nucleus and the cytoplasm of a plant cell (Lannoo and Van Damme, 2010).The majority of the known plant lectins are built up of one or more lectin-like domains coupled to un-related domains such as aerolysin, AIG1, chitinase, dirigent, F-box, Kelch, kinase, LRR, NB-ARC, PAG, or TIR domains (Van Damme*et al*., 2008).Based on the domain structure and binding capabilities, lectins are classified into the following seven categories (Vandenborre*et al*. 2011a). These categories include 1) The legume lectins, 2) The monocot mannose binding lectins, 3) The chitin binding lectins composed of hevein domains, 4) The type-2 ribosome inactivating protein (RIP), 5) The *jacalin* related lectins, 6) The *amaranthin* lectin family and 7) The *Cucurbitaceae* lectins.

Legume lectins,one of the largest and best characterized families of plant lectinsare homologous carbohydrate binding proteins that are found mainly in the seeds of legume plants. Some legume lectins are synthesized as prolectins in the endoplasmic reticulum and undergo post translational modifications in the Golgi apparatus to function as secretory proteins (Moreira *et al*. 2013). Concanavalin A (*Con*A) was the first plant lectin which was purified, crystallized and whose primary structure along with three dimensional structure were resolved (Sumner and Howell, 1936; Edelman *et al*.1972; Hardman and Ainsworth, 1972). However, the soybean seed lectin gene was the first to be sequenced (Vodkin*et al*. 1983).All legume lectins share similar three dimensional structures. The front face of the monomer is built from a curved seven-stranded â-sheet, whereas the back face is formed from a flat six-stranded â-sheet. They are interconnected by turns and loops and form flattened dome shaped structure, which is known as the “jelly-roll motif”, commonly found in viral coat proteins. Four loops located at the upper part of the dome form the monosaccharide binding site. Legume lectins contain almost no á-helix; hence belong to the class of all beta proteins (Sharon and Lis, 2002). The basic architecture of carbohydrate recognition domain (CRD constitute four binding site loops A, B, C, D, which are adjacent to eachother in the pocket. Theresidues in the binding pocket show greater variability and involved in specificity determination (Benevides*et al*. 2012). The floor of different loops contain some vital amino acidsresidues including Asp in Loop A, Gly or Arg (in *Concanavalia* and

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*Diocleal*ectins) in Loop B, Asn and an aromatic residue in Loop C, which contributeto hydrogen bonds and Vander Waals interactions with the sugar. The monosaccharide specificity is determined primarily by variation in loop C and D (Sharmaand Surolia, 1997; Rao*et al*. 1998). The CRD lies in close proximityof metal binding sites and require Ca^{2+} and transition metal ion Mn^{2+} for theirbinding activity (Etzler*et al*. 2009).

Interaction with the insect glycoprotein

Glycan array studies have revealed that many plant lectins have a strong affinity for glycoproteins that are predominantly present in insects.Often the lectins are obstinate to the digestive enzymes of insects and bind to surface glycans of gut epithelial cells or the secretory gut proteins causing physiological imbalance, resulting in toxicity. Although there are several reports on the lectin induced morphological and structural changes in the insect guts, yet the molecular mechanism of lectin induced toxicity has not been understood in detail (Zhu-Salzman*et al*. 1998; Fitches *et al*. 2001).Lectins with N-acetylglucosamine-specificity have a high affinity for chitin, the main constituent of the peritrophic matrix in the insect gut (Hakim *et al*., 2010). It has been suggested that certain lectins bind to the peritrophic matrix and damage its integrity, which in turn would break the protection given by the peritrophic membrane to the gut epithelium below. The N-acetylglucosamine-specific GSII lectin from *Griffonia simplicifolia* showed insecticidal properties against the cowpea weevil (*Callosobruchusmaculatus*) (Zhu *et al*., 2006). Similarly, Melander*et al*. (2003) recorded reduced growth in pollen beetle (*Meligethesaeneus*) larvae fed on transgenic oilseed rape expressing the mannose/glucose-specific Pisum sativum agglutinin (PSA, pea lectin).

Another enzyme that was targeted in the midgut of the pea aphid (*A. pisum*) by both GNA and ConA was identified as a membrane-bound aminopeptidase (Cristofoletti*et al*. 2006). ConA was found to bind primarily to the midgut andconsequently to the whole digestive tract, and caused morphological changes in theepithelium cells as well as increased secretion and detachment of the apical membrane. In contrast to earlier findings for the *Phaseolus vulgaris* agglutinin (PHA) in *Lygushesperus* (Hemiptera) (Habibi*et al*., 2000), no lysis of epithelium cells was seen. The changes inflicted by ConA-binding to gut surface receptors can result in changes in metabolism and cell function in the epithelium, which, through a feedback mechanism, may lead to altered feeding behavior. This was also shown for pest insects such as beetles, aphids and caterpillars, and for non target insects such as honeybees (Vandenborre*et al*. 2011)

Legume lectin monomer

Most legume lectins are present mainly as homodimersor homotetramers, with the tetramers being dimmers of dimers. The remarkable feature of legume lectins isthat although all the monomers have similar tertiary structures, butmodes of quaternary associations are very different.The different kinds of quaternarystructures present in legume lectins include Canonical,ECorL-type, GS4-type, DBL-type, ConA-type, PNA-type,GS1-type, DB58-type, and Arcelin-5-type. All thesearedimers or tetramers, except Arcelin-5, which exists as amonomer. Arcelin-5 and Arcelin- 1 (both from *Phaseolus vulgaris*), which show similarity to legume lectins in sequence and tertiary structure, are not lectins, because they do not bind sugars due to lack of crucial metalbinding residues. The dimers include Canonical, ECorL,GS4, and DB58 types and the tetramers include DBL,ConA, PNA, and GS1 types (Banerjee *et al*. 1994). Thesenine quaternary structures consist of seven different types ofdimeric interfaces, namely types II, X1, X2, X3 (handshake),X4 (back to back), and the unusual interfaces ofPNA and GS1 (Manoj and Suguna, 2001). The two unusualdimeric interfaces seen in PNA and GS1 are the only knowncases of such interfaces, and hence, the name “unusual.”Most of these quaternary associations occur by the diverseassociations of the flat six-stranded “back” â-sheet. TheCanonical mode of association, which is result of dimerization in legume lectins,occurs by the side-by-side arrangement of the back â-sheetto form a contiguous 12-stranded sheet. The other associations,namely ECorL, GS4, DB58, and ConA types, occurby the overlap of two back â-sheets on each other. Thedifference between these associations is the angle betweenthe sheets during overlap. The unusual associations of PNAand GS1 mainly involve the top â-sheet and loop regions.The X1 interfaces of the DB58 dimer and DBL tetramer arealso stabilized by a helix sandwiched between the twomonomers.Of about 50 legume lectin sequences that have been determined all show pairwise sequence identities not lower than 35%. Recently, it has been suggested that also in the animal kingdom legume lectin homologues may be present.Carbohydrate binding activity of legume lectins depends on the simultaneous presence of both aCa^{+2} and a transition metal ion Mn^{+2}. These metal binding sites were first studied for concanavalin A and have been found to be extremely well conserved in all other legume lectin structures.The legume lectin family also contains two proteins with no known carbohydrate recognition activity that do not possess the otherwise strictly conserved metal binding sites: the á-amylase inhibitorfrom *Phaseolus vulgaris* and arcelin, a seed defence protein.

Action mechanism of lectin

The entomotoxic activity of lectins relies upon interactions with different glycoproteins or glycan structures in insects mid gut, resultshampermetabolic activity of organism. However, lectins possess at least one carbohydrate-binding domain and different sugar specificities, which interact with variety of glycan structures in the insectsbody, possible targets for lectin binding are numerous (Figure 1) (Vandenborre*et al*., 2011). Therefore, it is difficult to predict the universal mode of action for each lectin and even more

difficult to understand the variability in insect toxicity upon exposure to different plant lectins. Ingestion of lectin by insects showed binding to the midgut tract causing disruption of the epithelial cells, elongation of the striated border microvilli, swelling of the epithelial cells into the lumen of the gut leading to complete closure of the lumen, permeability of cell membrane to allow the harmful substances penetrations from lumen towards haemolymph and impaired nutrient assimilation by cells, allowing absorption of potentially harmful substances from lumen into circulatory system, fat bodies, ovarioles and throughout the haemolymph. Thus, it could be concluded that the action mechanism of various lectins at the cellular levels of insects differs between different insect species. Although,insecticidalmechanisms of lectins at the cellular level are still not clearly elucidated yet and the information is scarce.

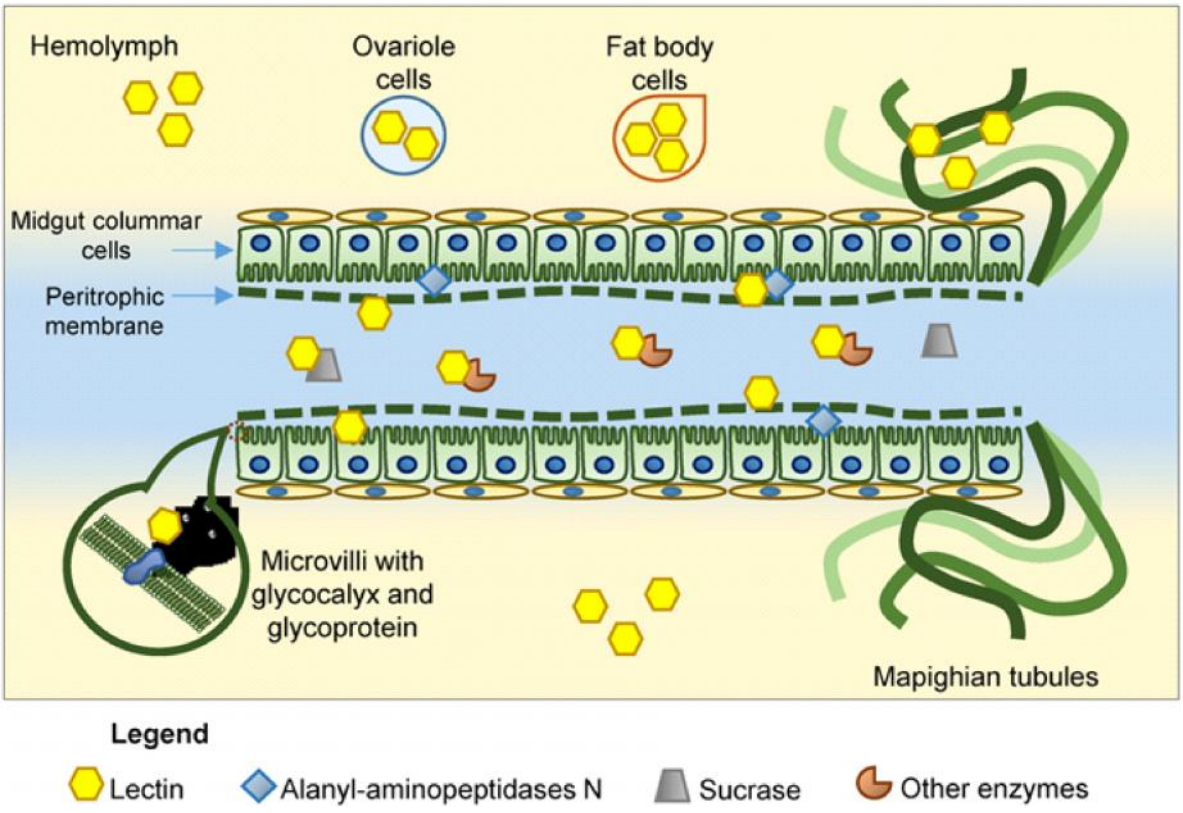

Fig.1 Representating scheme showing binding site for plant lectins (Macedo *et al.*, 2015)

Applications of Legume Lectins

Lectins have become the centre of attraction for biologists and in particular for the research and applications in agriculture and medicine (Movafagh *et al.*, 2013). Lectins have found use in diverse fields of biology and as more lectins are being isolated and their role in nature elucidated, they continue to occupy a vital place in agricultural and therapeutic areas of research.

Insecticidal Activity

One of the significant roles of lectins is in host defence against pathogens and predators (Hakim et al., 2010). Although the use of pesticides is quiet effective, their excessive and injudicious use has led to imbalances in agro-ecosystem. Development of resistance to insecticides has necessitated the application of higher dosages of the same pesticides or increased number of pesticide applications. This not only increases the cost of pest control, but also results in insecticidal hazards and pollution of the environment. Several alternative measures have been attempted including use of plant lectins. The anti-insect activity of plant lectins against a wide array of insect species have been well documented and represents a potential of using plant lectins as naturally occurring insecticidal agents against pests, which restrain increased crop production (Hogervorst *et al.*, 2006).

Lectins have been suggested as one of the promising agents against insect pests and have been engineered successfully into a variety of crops including wheat, rice, tobacco, and potatoes. This approach could be used as a part of integrated pest management strategies and caveat pest attack. Large-scale implementation of transgenic

insecticidal plants does not display considerable negative effects on the environment. Moreover, at least some transgenic plants can improve the corresponding environments and human health because their production considerably reduces the load of chemical insecticides and herbicides (Velkov*et al*., 2005).

Coleopteran insects of the family *Bruchidae*, the seed weevils have been associated with the seeds of leguminous plants through co-evolutionary processes. Lectins of the legume lectin gene family having specificity for N-Acetylglucosamine (GlcNAc) appear to function in plant defense against seed feeding coleopteran insects. Arcelin, a protein of the legume lectin gene family from wild P. vulgaris, is toxic to the Mexican bean weevil, *Zabrotessubfasciatus* (Osborn *et al*., 1988), and has been used to develop *Z. subfasciatus*-resistant common bean lines (Kornegay*et al*., 1993). Movafagh*et al*., 1996 have demonstrated the insecticidal activity of Lectin Gene from *Griffonia simplicifolia* (Leguminosae) against cowpea weevil. African yam bean lectin provides effective resistance against the cowpea weevil,*Callosobruchus maculates* (Machuka*et al*., 2000).

Pyramiding two or more resistance genes into the same plant, particularly those with completely different modes of action/target receptors, could be a useful strategy for achieving higher insecticidal activity, broader protective spectra and increased durability of transgenic plants (Gatehouse & Gatehouse, 1998; Roush, 1998). Zu-Salzman*et al*., 2003 achieved increased toxicity and durability against the coleopteran cowpea bruchid beetle (*Callosobruchus maculates*) by fusing soybean cysteine protease inhibitor soyacystatin N (scN) and *Griffoniasimplicifolia* legume lectin II (rGSII).

Antimicrobial activity

Lectins have Cytotoxic effects which is revealed by antitumoral and antiviral activities and also by deleterious effect on microorganisms. Lectins of different carbohydrate specificities are able to promote growth inhibition or death of fungi and bacteria. Upon *Trichodermaviride* infection in *Gastrodiaelata*, expression of lectins in the vascular cells of roots and stems gets induced (Sa *et al*. 2009), indicating lectin play an important role defense against fungi. Thus, lectins may be introduced into plants to protect them from fungal attack. Does *et al.,* 1999 introduced the gene encoding the precursor to stinging nettle (*Urticadioica*L.) isolectin I into tobacco (*Nicotiana tabacum*). In transgenic plants this precursor was processed to mature-sized lectin. In the transgenic plants the germination of spores of *Botrytis cinerea*, *Colletotrichumlindemuthianum*, and *T. viride* was significantly reduced.

Plant lectins can neither bind directly to glycoconjugates on the fungal plasma membranes nor penetrate the fungal cytoplasm because of the cell wall barrier. It is not likely lectins directly inhibit fungal growth by modifying fungal membrane permeability and/or structure. However, there may be indirect effects manifested due to binding of lectins to carbohydrates on the fungal cell wall surface. The antifungal activity of legume lectins is related to the lectin carbohydrate binding property (Damico *et al*., 2003), that might allow lectin molecules with binding activity towards certain carbohydrate components in the fungal cell wall affecting its activity and viability as most lectins recognize either N-acetylneuraminic acid, N-acetylglucosamine, N-acetylgalactosamine, galactose, mannose, or fucose in accordance with the conclusion of Lis and Sharon (1998). Alternatively, antifungal activity of some proteins or peptides was associated with chitin binding property and the active proteins should have a specific amino acid sequence and a cysteine/glycine rich chitin binding domain (Grenier*et al*., 1999). This chitin binding property might simulate the carbohydrate binding property as chitin is composed of modified glucose subunits (N-acetyl glucose amine) which can be equally recognized by lectin as glucose. Chitin binding leads to the disruption of the fungal cell wall that increases toxicity (Yun *et al*., 1998). Chitinase-free chitin-binding stinging nettle (*Urticadioica* lectin) inhibits fungal growth. Cell wall synthesis was hampered because of reduced chitin synthesis and/or deposition (Van Parijs*et al*., 1991). The effects of nettle lectin on fungal cell wall and hyphal morphology suggest that the nettle lectin regulates endomycorrhizal colonization of the rhizomes. Severa1 other plant lectins inhibit fungal growth. The first group includes small chitin-binding merolectins with one chitin-binding domain, e.g., hevein from rubber tree latex (Van Parijs*et al.,* 1991) and chitin-binding polypeptide from *Amaranthuscaudatus* seeds (Broekaert*et al*., 1992). The only plant lectins that can be considered as fungicidal proteins are the chimerolectins belonging to the class I chitinases. However, the antifungal activity of these proteins is ascribed to their catalytic domain.

The antifungal activity of legume lectins have been well characterised against*Fusariumoxysporum*, *Aspergillusfl avus*, and *Trichodermsviride* (Yan *et al*., 2005; Ciopraga*et al*., 1999; Ye *et al*., 2001; Peumans and Van Damme, 1995; Sitohy*et al*., 2007). Purified legume lectin from *Archidendronjiringa* inhibits growth of plant pathogenic fungi, Colletotrichumcassiicola, Exserohilumturcicum and Fusariumoxysporum (Charungchitrak*et al*., 2011)

Many human pathogens utilize cell surface glycans as either receptors or ligands to initiate adhesion and infection (Sharon and Lis, 1989; Sharon and Lis, 2003; Zem et al., 2006; Hyun et al., 2007; Oppenheimer et al., 2008; Magalhaes et al., 2009; Mukhopadhyay et al., 2009). *Escherichia coli* (*E. coli*), for example, binds to host mannosides, while influenza virus binds to host sialic acids (Mukhopadhyay et al., 2009). Other strains of *E. coli* have been discovered that demonstrate specificities towards other host cell surface carbohydrate moieties such as galabiose (Gal-á-4-Gal) and NeuAc-á-2,3-Gal-â-3- GalNAc (Khan et al., 2000; Buts et al., 2003). The genital pathogen

*Neisseria gonorrhea*specifically binds N acetyl lactosamine (Gal-â-4-GlcNAc, LacNAc), and *Streptococcus pneumonia* specifically binds the pentasaccharideNeuAc-á-3-Gal-â-4-GlcNAc-â-3-Gal-â-4-Glc as well as the internal tetra- and trisaccharides Gal-â-4-GlcNAc-â-3-Gal-â-4-Glc and GlcNAc-â-3- Gal-â-4-Glc respectively. *Pseudomonas aeruginosa*specifically binds fucose (L-Fuc) (Barthelson et al., 1998). Bacteria can discriminate between two identical glycans that differ in only one hydroxyl group (Sharon, 2006). Antibacterial activity on Gram-positive and Gram-negative bacteria occurs through the interaction of lectin with components of the bacterial cell wall including teichoic and teicuronic acids, peptidoglycans and lipopolysaccharides. The purified legume lectin from *Archidendronjiringa* shows antagonastic action against both gram positive and gram negative bacteria like *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*and *Candida albican* (Charungchitrak*et al*., 2011).

Antitumor activity

It is well known that lectins have an antitumor effect. Plant lectins signify a well-defined and a novel source of anticancer compounds. A number of plant lectins (predominantly galactoside and galNAc specific) have been in pre-clinical and clinical trials as potential drugs for treatment of cancer (Ernst *et al*., 2003). Small glossy black soybean (*Glycine max*) lectin impeded proliferation of breast cancer MCF7 cells and hepatoma HepG2 cells (Lin *et al*. 2008).Leguminous lectins from *Canavaliaensiformis*(ConA) and *Ulexeuropaeus*(UEA-1) were used as histochemical markers for parotid gland mucoepidermoid carcinoma with low, intermediate, and high grade dysplasia. ConA localization in the cytoplasm and/or plasma membrane was significantly associated with neoplastic cells from the three grades of severity, whereas UEA-1 was associated with low and intermediate grade dysplasia (Sobral*et al*., 2010).

Biotechnological Applications

Galanthus nivalis agglutinin, or GNA, is composed of four identical (12 kDa) subunits with affinity for á-1,3- or 1,6-linked D-manose residues in carbohydrates (Van Damme et al., 1987)[71]. Artificial diet bioassays revealed insecticidal effects of GNA for several insects, including sap-sucking insects. Transgenic rice plants indicating tissue-specific expression of GNA were constructed and evaluated for control of the brown plant hopper (Rao et al., 1998)[43]. Insects fed on transformed plants exhibited a significant decrease in survival and fecundity, which reduces their growth. GNA also exhibited insecticidal effects for the glasshouse potato aphid (*Aulacothumsolani*) through transformation of potato plants (Down et al., 1996)[72]. GNA expression level of 0.3%–0.4% provoked a significant reduction in both survival and fecundity in glasshouse bioassays. The pea lectin (P-Lec) and cowpea trypsin inhibitor (CpTI) were expressed in tobacco plants which provide resistance against the tobacco budworm (*Heliothisvirescens*) (Boulter et al., 1990)[74]. Plants expressing P-Lec (C"L+) or CpTI (C+L") only, tobacco plants expressing both defense genes (C+L+) showed a higher mortality rate and the lowest larval weight. In contrast with control, transgenic plants showed markedly reduced leaf damage.

Novel applications

Lectins from *Viciacracca* seeds react specifically with human blood group A erythrocytes (Rüdiger 1977). These lectins may be considered as excellent reagents for anti-A, anti-B, anti-N etc., but the anti-A and anti-M antisera are not yet have commercially significance. Lectin from *Dolichus biflorus* can be used as anti-A, and lectin from *Griffonia simplicifolia* as anti-B. Lectin from *Viciagraminea* is said to be a good typing reagent as anti-N (Khan *et al*. 2002).Concanavalin A can be used for immobilization in lectin affinity chromatography for isolation of glycopeptides that express biantennary and hybrid N-linked structures and high mannose glycans, which are abundant in both embryonic stem cells and embryo bodies stages (Alvarez-Manilla*et al*. 2010).

Conclusion

Lectins are a subject with ennormous potential and of intense investigations. As more lectins are isolated and further studies are conducted on the biological activities and mechanisms of action of lectins, the production of lectins can be improved and new applications of lectins can be found and explored for noteworthy contributions in various fields of biology.

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