Plant lectins: an overview

Lectinas de planta: uma revisão

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ABSTRACT

Lectins are involved in a range of biological mechanisms related to recognition and binding to carbohydrates. This ability is possible due to the presence of a carbohydrate recognition domain with capacity to differentiate between various oligosaccharides without modify the molecules which it binds. The study of lectins, inserted in the field of Glycobiology, started with Stillmark’s work with castor bean extract and continues until today with the use of advanced techniques such as robust hemagglutination assay and affinity chromatography. Lectins are particularly abundant in plants where seem to be involved in seed maturation or dormancy, plant defense, storage material, and N2 fixation. Furthermore, lectin plants show immunomodulatory effects through signal-transduction system and consequent cytokines production, a research field founded by Ehrlich and his studies with mice immunization. Here we focus on plant lectins and their role in plant physiology, immune response, and structural analysis.

Keywords: Lectin; Agglutinin; Hemagglutinin; Lectin-like proteins;
RESUMO

As lectinas estão envolvidas em uma variedade de mecanismos biológicos relacionados ao reconhecimento e ligação a carboidratos. Essa capacidade é possível devido à presença de um domínio de reconhecimento de carboidratos que reconhece, de forma diferenciada, vários oligossacarídeos sem modificar as moléculas às quais se liga. O estudo das lectinas, inserido na área da Glicobiologia, começou com o trabalho de Stillmark com extrato de mamona e continua até hoje com o uso de técnicas avançadas como o ensaio de hemaglutinação robusta e a cromatografia de afinidade. As lectinas são particularmente abundantes em plantas onde parecem estar envolvidas na maturação ou dormência das sementes, defesa da planta, armazenamento e fixação de N2. Além disso, lectinas de plantas apresentam efeitos imunomoduladores através do sistema de transdução de sinal e consequente produção de citocinas, campo de pesquisa fundado por Ehrlich e seus estudos com imunização de camundongos. Aqui nos concentramos nas lectinas vegetais e seu papel na fisiologia vegetal, resposta imune e análise estrutural.

Palavras-chave: Lectina; Aglutinina; Hemaglutinina; Proteínas semelhante a lectinas;

List of abbreviations
ConA Concanavalin A
CRD Carbohydrate recognition domain
MHC Major histocompatibility complex
PNA Peanut agglutinin
SalT Salt-inducible protein (SalT)
SBA Soybean
TLR Toll-like receptor
INTRODUCTION

Lectins are widespread proteins in all kingdoms of life, particularly abundant in plants, with the ability to recognize and bind to free carbohydrates or glycoconjugates in a reversible and highly specific manner (TSANEVA, M.; VAN DAMME, 2020). Erythrocyte-agglutinating ability was the first property of lectins used to identify them in plant extracts and biological assays. The finding that these proteins selectively agglutinated erythrocytes of particular human blood group within the ABO system leads to the introduction of the term ‘lectin’, derived from the Latin verb for “to select” (RENKONEN, 1948). However, the existence of monovalent lectins created the need to extend this concept, becoming an increasingly less restrictive one. Hence, plant lectins are defined as “all plant proteins that possess at least one noncatalytic domain that binds reversibly to a specific mono- or oligosaccharide” (PEUMANS and VAN DAMME, 1995). Plant lectins three-dimensional structure is characterized by β-sheets connected by α turns, β turns and bends (LAGARDA-DIAZ et al. 2017). The binding ability is possible due to the presence of a carbohydrate recognition domain (CRD), a globular structure of fewer than 200 aminoacids (WEIS and DRICKAMER, 1996).

To these organisms, they compound a set of physiological important molecules. They have been implicated in several roles in dealing with biological function in seeds and plant defense mechanism. In plant physiology, seems to be related to seed maturation or germination, being synthesized and storage during seed development, and broken during germination and seedling growth, to provide amino acids for the growing seedling. In plant defense, had been proposed that lectins may protect plants against pathogens during seed imbibition, germination, and early growth of the seedlings (DE CONINCK and VAN DAMME, 2021).

Brief historical background

Lectin proteins were first described in 1888 by Stillmark in his doctoral thesis, who observed that the seed extract of castor bean (Ricinus communis) could agglutinate erythrocytes (FRANZ, 1988). In 1891, Hellin identified a toxic hemagglutinin present in bean seed extract of Abrus precatorius. These lectins were used by Ehrlich (1899), a pioneer in immunological research, to immunize mice. This researcher has established
some of the basic principles of immunology and showed that plants agglutinins could be antigenic models more useful than the bacterial toxins (EHRLICH, 1899).

Elfstrand (1989) introduced the term hemagglutinin, *Blutkörperchenagglutinin* in German, to describe this group of proteins capable of causing cell agglutination. Between 1907/1909, Landsteiner and Raubitschek detected non-toxic agglutinins in plants and were the first to discuss specificity of lectins proteins. Moreover, they observed that legume seed extracts show different hemagglutination properties when combined with different animal erythrocytes and a “deagglutination” property by hog gastric mucin (GABIUS, 2013).

The ability of agglutination by lectins due to its binding to carbohydrates it has only been demonstrated in 1952 by Watkins and Morgan, during their study about the presence of sugars on erythrocytes surfaces and their potential roles as identity markers of cells, a central idea in glycobiology (WATKINS and MORGAN, 1952). The ability of plant agglutinins to discriminate between erythrocytes of different blood types led Boyd and Shapleigh (1954) to use the term lectin.

The structural characterization of lectins started with Summer and Howell (1936) in their study on concanavalin A (ConA), the hemagglutinin from the jack bean, a pioneering protein in the study of lectins (GABIUS, 2013). ConA, until today, is used as a started point to another analysis in order to characterize lectins, using robust hemagglutination assay and affinity chromatography, for the screening of activity and purification of these proteins (SUMNER and HOWELL, 1936).

Lectin classification and occurrence

To be qualify as a lectin there are three requirements for a protein: i) contain a CRD which sole purpose is the effective differentiation between various oligosaccharides; ii) is not the product of the immune system like antibodies are and iii) biochemically does not modify the carbohydrate moiety which it binds, different than carbohydrate-specific enzymes – like glycosyltransferases, glycoside hydrolases and transglycosylases, that modify their substrates (GOLDSTEIN et al. 1980; MISHRA et al. 2019).

The classification of these proteins is based on the overall domain architecture and properties of agglutination, subdivided into constituting four groups: merolectins, hololectins, chimerolecctins, and superlectins (TSANEVA, M.; VAN DAMME, 2020).
Merolectins have a single binding domain carbohydrate. They are simple polypeptide proteins, which due to their monovalent structure are unable to precipitate glycoconjugates or agglutinate cells. Hololectins present two or more homologous or identical CRDs. This group comprises all lectins that have multiple binding sites to the same sugar or structurally similar sugars and, because of this, are able to agglutinate cells or precipitate glycoconjugates. The chimerolectins are composed of one or more CRDs tandemly arrayed with an unrelated domain with catalytic or biological activity, which acts independently of the carbohydrate binding domain. Depending on the number of CRDs, these molecules may or not show hemagglutination activity. Superlectins are a special class of hololectins, which have two or more CRDs with specificity for structurally different sugars (TAYLOR et al. 2022). Lectins represent a diversified group of proteins with respect to size, composition, and structure (SHARON and LIS, 1987). Each lectin domain has its own characteristic folding with one or more carbohydrate-binding sites. In general, the three-dimensional structure of lectins is composed of a high content of $\beta$-sheets with little contribution from $\alpha$-helices. The $\beta$-sheets are connected by loops forming antiparallel chains (SHARON, and LIS, 1990). A ribbon drawing gallery of the crystal and solution structures of representative fold of lectins or lectin-like proteins from plants is shown in figure 1. Different classification systems have been used in an attempt to categorize this heterogeneous group of proteins. In principle, they were grouped based on the carbohydrate binding specificity (PEUMANS and VAN DAMME, 1995), but this system proved to be artificial and uninformative from an evolutionary point of view (DE SCHUTTER, K.; VAN DAMME, 2015). With the progress in the purification and characterization of lectins, evidence was accumulated that lectins are a very heterogeneous group of proteins, artificially grouped based on the capability of cell agglutination.

**Figure 1** – Ribbon drawing gallery of representative fold of lectins or lectin-like proteins from plants. Three-dimensional structures of lectins were extracted from the Protein Data Bank. Some lectins distributed in several kingdoms share the same protein motifs, e.g. R-type, the $\beta$-Trefoil motif that is present in animal, plant, fungal, bacterial and viral lectin. (A) ACA-like (PDB 1JLX); (B) Chitinase-like (PDB 1E9L); (C) CV-N family (PDB 2C4D); (D) F-type (PDB 1K12); (E) ABL-like (PDB 1Y2U); (F) Hevein family (PDB 1EHH); (G) H-type (PDB 3O0W); (H) Jacalin family (PDB 1JAC); (I) Legume family and L-type-like (PDB 1LOG); (J); LNP-
type (PDB 3CJA); (K) LysM family (PDB 4B8V); (L) Monocot family (PDB 1MSA); (M) M-type (PDB 1X9D); (N) Ricin-B family (PDB 2AAI).

Recently, biochemical and transcriptome analysis revealed that lectins can be divided into multiple families of structurally and evolutionarily related proteins based on a conserved CRD. In plants, lectins were classified into twelve families (VAN DAMME et al. 2008), but with new findings of the three-dimensional structure, today is about fifteen (FUJIMOTO et al. 2014) – Table 1. However, there are many lectins that cannot be included in this classification system because they have a unique structure that can belong to others well-established protein families that are generally not linked to sugar-binding activity (DE SCHUTTER, K.; VAN DAMME, 2015).
Table 1 – Lectins families classification based on evolutionary and structurally related lectin domains †

<table>
<thead>
<tr>
<th>Plant lectin family</th>
<th>Fold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amaranthin family (ACA-like)</td>
<td>β-Trefoil</td>
</tr>
<tr>
<td>Chitinase-like</td>
<td>(β/α)8-barrel</td>
</tr>
<tr>
<td>CV-N family</td>
<td>(3-stranded β-sheet and β-hairpins)2</td>
</tr>
<tr>
<td>F-type (AAA-like, Eel-lectin, fucolectins)</td>
<td>β-Sandwich with Ca ion</td>
</tr>
<tr>
<td>Fungal fruit-body (Actinoporin-like, ABL-like)</td>
<td>α/β-Sandwich (actinoporin-like)</td>
</tr>
<tr>
<td>Hevein family</td>
<td>Hevein-like cystine knot motif Dimer</td>
</tr>
<tr>
<td>H-type</td>
<td>Six-stranded antiparallel β-sandwich</td>
</tr>
<tr>
<td>Jacalin related family</td>
<td>β-Prism I</td>
</tr>
<tr>
<td>Legume family (L-type)</td>
<td>β-Sandwich</td>
</tr>
<tr>
<td>LNP-type (N-type)</td>
<td>RNAseH-like α/β-fold</td>
</tr>
<tr>
<td>L-type-like</td>
<td>β-Sandwich</td>
</tr>
<tr>
<td>LysM family (bulb-type lectin)</td>
<td>LysM βααβ-fold (lysin motif)</td>
</tr>
<tr>
<td>Monocot family (bulb-type lectin)</td>
<td>β-Prism II</td>
</tr>
<tr>
<td>M-type</td>
<td>(α/α)7 –barrel</td>
</tr>
<tr>
<td>Ricin-B family (R-type)</td>
<td>β-Trefoil</td>
</tr>
</tbody>
</table>

†Lectin families, such as X-lectin, Nictaba-like lectin, and Euonymus europaeus lectin, whose members’ structures have not been determined, are not included in this table.

Source: Adapted from VAN DAMME et al., 2008; FUJIMOTO et al., 2014

In plants, these proteins are predominantly isolated from seeds, mostly of legume plants, where they constitute about 10% of the total soluble protein of the seed extracts (INGALE and HIVRALE, 2013). In legume plants, they have an interesting feature due to their oligomeric structure, forming dimers or tetramers, which is directly related to their potential to agglutinate cells and to precipitate multivalent carbohydrates (TAYLOR et al. 2022). Furthermore, lectins are also found in vegetative tissues such as leaves, fruits, roots, tubers, rhizomes, bulbs, bark, stem, phloem sap and even nectar (PEUMANS and VAN DAMME, 1995).

Constitutively Expressed and Inducible Lectins

The lectins of constitutive expression are known as “classical lectins” and are expressed in high amounts in seeds and vegetative storage tissues. Most of the constitutively expressed lectins are synthesized with a signal peptide, and accumulated in vacuoles or related organelles, or are secreted to the extracellular compartment (KATOCH and TRIPATHI, 2021). It is generally believed that the storage of proteins like lectins has multiple functionalities (CÂNDIDO et al. 2011). In normal conditions,
Lectins remain inactive as storage proteins, being released when the plant is invaded and its cellular contents disrupted by microorganism or insect. The stored lectin may agglutinate or sicken the pathogen or predator, acting as defense proteins (PEUMANS and VAN DAMME, 1995; DE CONINCK and VAN DAMME, 2021; JAIN et al. 2022).

In addition, lectin expression also can be induced as particular responses toward specific biotic/abiotic stimuli, such as salt stress, drought, light, heat or cold shock, wounding or treatment with abscisic acid, jasmonic acid, and gibberellins (LANNOO and VAN DAMME, 2014). In the absence of plant stress, the inducible lectins generally are not expressed at detectable levels. Most strikingly, all inducible lectins identified thus far reside in the nucleus and the cytoplasm of the cells (LANNOO and VAN DAMME, 2010). Based on these observations, there is a consensus that protein-carbohydrate interactions lectin-mediated in the cytoplasm and the nucleus play an important or possibly even crucial role in the stress physiology of the plant cell (VAN DAMME et al. 2004). During the last decade, this new class of plant lectins has been extensively studied (MACEDO et al. 2015). It has been proposed that lectins located in the cytoplasm and nucleus could act as decoders (VAN DAMME, 2022). For example, the stress-inducible lectin from tobacco, called Nictaba, is present in the nucleus and has been shown to interact with O-GlcNAc-modified histones. Thus, it can be hypothesized that, under stress conditions, Nictaba could modulate gene expression through chromatin remodeling (DELPORTE et al. 2014a; DELPORTE et al. 2014b). The possibility of inducing the expression of defense genes was also suggested by the experiments by Moradi et al. (2021). The expression of the lectin Coprinopsis cinerea lectin 2 (CCL2) in Arabidopsis, made the plants more resistant to fungal pathogens, including Botrytis cinerea, and the phytopathogenic bacteria Pseudomonas syringae, however, the protective effect of CCL2 does not seem to be direct, since the lectin was not able to inhibit the growth of B. cinerea in in vitro assays. The authors then propose that this lectin acts through the induction of defense genes related to jasmonic acid and salicylic acid signaling (MORADI et al. 2021). Based on these observations, the concept was developed that lectin-mediated protein-carbohydrate interactions in the cytoplasm and nucleus play an important or possibly crucial role in plant cell stress responses (VAN DAMME et al., 2004, VAN DAMME, 2022). The first inducible lectin to be purified was a mannose-specific jacalin-related lectin (called Orysata) from NaCl-treated rice seedlings (ZHANG et al. 2000). Orysata was designated as a lectin only in 2000. However, ten years earlier Claes et al. (1990)
showed that Orysata is a salt-inducible protein (SalT), and the salT mRNA accumulates in sheaths and roots of mature plants and seedlings upon salt or drought stress. SalT expression was also induced by abscisic and jasmonic acid treatment (MOONS et al. 1997).

Physiological role of plant lectins

In the past, lectins were widely used as specific tools in studies of carbohydrates chemistry and histology. However, in recent years they have been recognized as a class of communication proteins involved in interactions between plants and their environment and therefore, can play an important role in plant physiology (VAN DAMME et al. 1998). This question of the possible physiological role of lectins has intrigued researchers since 1970’s (ETZLER, 1986). In spite of the extensive knowledge about the molecular biology and structure of lectins, their physiological role with respect to the plant is still not well understood (DE HOFF et al. 2009).

The first concepts proposed that lectins could function as antibodies to protect plants against harmful soil bacteria, control seed germination, or be involved in the transport and storage of sugars, but no evidence to confirm these propositions were found (VAN DAMME et al. 1998). Biological events such as seed maturation or maintenance of seed dormancy (PEUMANS and STINISSEN, 1983), mitogenic stimulators of plant embryonic cells, packaging or mobilization of storage materials WEBER and NEUMANN, 1990) and N2 fixation and source for developing embryo (PEUMANS and VAN DAMME, 1995; DÍAZ et al. 1995) have been associated to lectins.

Lectins are able to interact with foreign organisms through the glycoconjugates present on their surface or in the digestive tract (VAN DAMME et al. 1998). This discovery leads to an advance in the understanding of the physiological role of plant lectin beyond its acting in plant itself. Many hypotheses have been proposed over the years. One of them is that lectins protect plants against phytopathogenic microorganisms and insects as well as herbivores (SHARON and LIS, 2004).

The first evidence that reinforce the idea that lectins could act as plant defense proteins was based on the observation that WGA, peanut agglutinin (PNA), and soybean (SBA) lectins inhibited the sporulation and growth of fungi such as Trichoderma viride, Penicillium notatum, and Aspergillus niger (BARKAI-GOLAN et al. 1978). Posteriorly, the potato lectin also showed a similar effect on the phytopathogen fungal Botrytis cinerea.
(CALLOW, 1977) and the anti-fungal properties of several others lectins has been shown (GUAN and RAMALINGAM, 2008; VAN DEENEN et al. 2011; WANG, et al. 2012).

Lectins also act in the mechanisms of plant defense against insect herbivores. The insecticidal action of lectin found in black bean was reported in 1976. The feeding of bruchid beetles with a diet containing this lectin resulted in the death of their larvae, demonstrating a possible role of lectins in legumes that could be protective for them from attack by insect seed predators (JANZEN et al. 1976). Several other lectins were shown to be insecticidal. Among them WGA, *Galanthus nivalis* lectin and jacalin (SHARON and LIS, 2004), and the genes encoding *Allium sativum* leaf and bulb lectin (SADEGHI et al. 2008), *Galanthus nivalis* agglutinin (NAGADHARA et al. 2004), and pea lectin (MELANDER et al. 2003) have been introduced into tobacco, wheat, and rice to reduce predation by insects.

In a study with *Oryctes*, a mannose-specific lectin from the jacalin-related family, was expressed in transgenic tobacco and its insecticidal activity against three pest insects: beet armyworm (*Spodoptera exigua*), green peach aphid (*Myzus persicae*) and pea aphid (*Acrhythosiphon pisum*) were evaluated. *S. exigua* larvae fed on transformed plants showed higher mortality, reduced weight and extension of larval development. Similar effects also were observed in *M. persicae* and *A. pisum* (AL ATALAH et al. 2014).

However, despite advances in understanding the role of lectins to the physiology of the plant, other toxicity mechanisms, as resistance to plant bacterial and fungal pathogens lectin-associated, remain poorly defined.

Interaction between plant lectins and animal immune system

It is well documented that lectins show immunomodulatory effects that are initiated by their interaction with glycan’s moieties on the surface of immune cells. Such interaction initiates a signal-transduction system, which culminates in the cytokines production and induces efficient immune responses against tumors or pathogens infections. As an example, C-type lectins (CLR) can act as mannose, galactose or N-acetylglactosamine receptors, which have been shown to be involved in antigen internalization, processing and subsequent presentation of glycopeptides to T lymphocytes, via MHC class II. CLR recognize not only pathogen-associated molecular patterns (PAMPs) but also damage-associated molecular patterns (DAMPs) to promote innate immune responses and affect adaptive immune responses (LI et al. 2022). *In vitro*
and *in vivo* studies have shown that lectins are able to interact with proteins related to the immune system, improve pathogen recognition, increase phagocytosis, and promote the release of cytokines and other immune effectors (CHEN et al. 2021). Some lectins induced lymphocyte proliferation such as ConA (KILPATRICK, 1999). Others are able to stimulate the innate immune response, as soluble lectins that direct the elimination of the foreign agent and help in the phagocytosis by macrophages and dendritic cells. These proteins bind to oligomannosides of infectious microorganisms, causing activation of complement without the participation of antibody, and subsequent lysis of the pathogens, thus acting in innate immunity (GHAZARIAN et al. 2011). Furthermore, lectins present on the cell surface of dendritic cells and macrophages are involved in the immune surveillance, as endocytic receptors, and of cell signaling. Lectins type C can act as mannose, galactose or N-acetyl-galactose receptors, which are involved in the internalization of antigens, processing and subsequent presentation to T lymphocytes as a peptidemajor histocompatibility complex (MHC) (AARNOUNDSE et al. 2006; DENDA-NAGAI et al. 2010). This antigen presentation activates specific immune responses, leading lectins to participate indirectly in the adaptive immune system (GORELIK et al. 2001).

Therefore, these proteins are also directly involved in adaptive immunity. Leukocytes express L-selectins, which aid in the lymphocyte homing and trafficking of leukocyte to sites of inflammation (TEDDER et al. 1995). They mediate the binding of leukocytes to endothelial cells and thereby initiate a rolling phase, in which the lectins interact transiently with glycan ligands, leading eventually to their extravasation. The T lymphocytes once activated have reduced L-selectin expression or lacking altogether, allows them to migrate and exit at the site of inflammation via high-affinity interaction between integrin and their specific ligands (GORELIK et al. 2001). Another mechanism of action of the lectins as agonists of the immune system was proposed by Coltri et al. (2008). It occurs via Toll-like receptor (TLR). The Th1 cytokines production is induced by plant lectins through interaction with glycosylated receptors on macrophages and/or dendritic cells, such as type 2 and 4 Toll-like receptors (TLR2 and TLR4). Several plant lectins may act as TLR agonists. The SBA, PNA, ConA, and PHA lectins (PHA-L and PHA-P) stimulate extracellular TLRs: TLR-4 for SBA and PNA; TLR-2/6 for ConA; TLR- 2/6, -4 and for PHA-L, whereas WGA is able of activating all tested receptors, except TLR-3 and -4 (UNITT and HORNIGOLD, 2011). Plant lectins can also regulate
Th2 immunity. The ScLL, a lectin from *Synadenium carinatum* reduces the leukocyte trafficking and activates Th2 cytokine production in mice (ROGERIO et al. 2007). This type of response can be induced by the lectin B-chain of type-2 RIP from *Ricinus communis* through binding to D-galactose containing glycans present on the surface of enterocytes (CARTER et al. 2010).

**SUMMARY AND FUTURE OUTLOOK**

The analysis of lectins and glycans of a given cell or organism founded a field called Lectinology, inserted in the Glycobiology studies. The demand for development of drugs against acute and chronic diseases has propelled the Lectinology field forward through methodological advances in protein and carbohydrate chemistry, physical chemistry and gene technology (TSANEVA and VAN DAMME, 2020). The study of glycans as biomarkers as well as lectin engineering approach, supported by the recent advances in bioinformatics, are in the main direction of this field. In addition, the design of glycoarrays is expected to boost lectins to a more practical use in medicine, biology and another research areas.

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REFERENCES


WANG, Q.; ZHANG, Y. et al. Purification, characterization of a CkChn134 protein from Cynanchum komarovii seeds and synergistic effect with CkTLP against Verticillium dahliae. Protein Sci. v. 21, n. 6, p.865-875, 2012.

