

PLANT LECTINS, CHEMICAL AND BIOLOGICAL ASPECTS

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Lectins, carbohydrate-binding proteins of non-immune origin, that agglutinate cells or precipitate polysaccharides and glycoconjugates, are well distributed in nature, mainly in the Plant Kingdom. The great majority of the plant lectins are present in seed cotyledons where they are found in the cytoplasm or in the protein bodies, although they have also been found in roots, stems and leaves. Due to their peculiar properties, the lectins are used as a tool both for analytical and preparative purposes in biochemistry, cellular biology, immunology and related areas. In agriculture and medicine the use of lectins greatly improved in the last few years. The lectins, with few exceptions, are glycoproteins, need divalent cations to display full activity and are, in general, oligomers with variable molecular weight.

Although the studies on lectins have completed a century, their role in nature is yet unknown. Several hypotheses on their physiological functions have been suggested. Thus, lectins could play important roles in defense against pathogens, plant-microorganism symbiosis, cell organization, embryo morphogenesis, phagocytosis, cell wall elongation, pollen recognition and as reserve proteins.

A brief review on the general properties and roles of the lectins is given.

Key words: lectins – plant lectins – properties of lectins

Lectins are carbohydrate-binding proteins of non-immune origin that agglutinate cells and glycoconjugates and are capable of specific recognition and reversible binding to carbohydrates and sugar containing substances, without altering covalent structure of any glycosyl ligands (Goldstein et al., 1980; Kocourek & Horejsi, 1983; Liener et al., 1986).

Widely distributed in nature, the lectins can be found in almost all living organisms from plants to animals (vertebrates and invertebrates) and, even, microorganisms (for reviews, see Jaffé, 1969; Toms & Western, 1971; Gold & Balding, 1975; Goldstein & Hayes, 1978; Lis & Sharon, 1981; Pusztai et al., 1983; Etzler, 1985; Pusztai, 1989; Sharon & Lis, 1989).

The study of the lectins began with the work of Hermann Stillmark (1888) who, for the first time, observed that seed extracts (*Ricinus communis*) could agglutinate red blood cells. After this pioneer study, several theses and papers were published.

The utilization of affinity chromatography for the isolation of lectins, used for the first time by Agrawal & Goldstein (1965), greatly contributed to the development of the field. With the pure proteins, it was easier to study the lectin properties and their interactions with several systems. Table I shows some milestones in the history of lectin investigation (Gold & Balding, 1975; Kocourek, 1986; Sharon & Lis, 1989).

The simplest way to detect a lectin is to examine its ability to agglutinate erythrocytes or to precipitate glycoconjugates. The hemagglutinating activity can be enhanced, in some cases, by treating the cells with proteolytic enzymes and neuraminidase. For a better characterization of the lectin, however, it is essential to determine whether it is specifically inhibited by mono- or oligosaccharides. This specificity is usually determined by hapten inhibition techniques, comparing the sugars on the basis of the minimum concentration to inhibit hemagglutination or precipitation reactions.

TABLE I

History

1884	Warden & Waddel/ Bruyllants & Venneman	Toxicity in <i>Abrus precatorius</i> seed extracts
1886	Dixson	Toxicity in <i>Ricinus communis</i> seed extracts
1888	Stillmark	Hemagglutinating activity in <i>Ricinus communis</i> seed extracts Toxicity in <i>Croton triglium</i> seed extracts
1890	Power & Cambier	Toxicity in <i>Robinia pseudoacacia</i> seed extracts
1890	Erlich	Use of abrin and ricin in immunological research
1891	Hellin	Hemagglutinating activity in <i>Abrus precatorius</i> seed extracts
1893	Siegel	Hemagglutinating activity in <i>Jatropha curcas</i> seed extracts
1897	Elfstrand	Hemagglutinating activity in <i>Croton triglium</i> seed extracts Introduction of the term hemagglutinin
1899	Camus	Hemagglutinating activity in snail (<i>Helix pomatia</i>)
1902	Landsteiner	Reversibility of the hemagglutination by heat
1902	Kauss	Inhibition of the hemagglutinating activity by non immune serum
1903	Noguchi	Hemagglutinating activity in horseshoe crab (<i>Limulus polyphemus</i>)
1907	Landsteiner & Raubitschek	Hemagglutinating activity in non toxic plants (<i>Phaseolus</i> , <i>Pisum e Lens</i>)
1908	Wienhaus	Agglutination of leucocytes and kidney and liver cells by <i>Phaseolus vulgaris</i>
1908	Landsteiner & Raubitschek	Species specificity of plant hemagglutinins
1909	Mendel	Hemagglutinating activity in <i>Robinia pseudoacacia</i> seed extracts
1909	Landsteiner	Inhibition of the hemagglutinating activity by heat treated serum
1909	Landsteiner & Raubitschek	Inhibition of the hemagglutinating activity by mucin
1912	Schneider	Hemagglutinins and germination
1917	Osborn & Mendel	Thermoinactivation of soybean toxic factors
1919	Sumner	Isolation and crystalization of Concanavalina A (Con a)
1926-7	Marcusson-Begun/Siever	Aplicability of lectins for blood typing
1935	Sugishita	Specificity of eel serum agglutinins
1936	Sumner & Howell	Sugar specificity of Concanavalin A
1947-9	Boyd & Reguera/Renkonen	Blood group specificity of plant hemagglutinins
1949	Liener	Toxicity of <i>Phaseolus vulgaris</i> hemagglutinins
1949	Jaffé	Thermoinactivation of <i>Phaseolus vulgaris</i> hemagglutinins
1952	Watkins & Morgan	Inhibition of lectins by simple sugars Demonstration with the aid of lectins that sugars are determinants of blood group
1954	Boyd & Sharpleigh	Introduction of the term lectin
1960	Nowell	Mitogenic stimulation of lymphocytes by the <i>Phaseolus vulgaris</i> lectin
1963	Aub	Agglutination of malignant cells by lectins
1964	Muelenaere	Parallel inactivation of hemagglutinating and antinutritional activity by heat
1965	Agrawal & Goldstein	Affinity chromatography for lectin purification
1966	Boyd	Lectins in algae
1970	Apsberg et al.	Use of Con A for affinity purification of glycoproteins
1974	Ashwell & Morel	Role of animal lectins in endocytosis of glycoproteins
1976	Gallo	Interleukin 2 discovered in medium of lectin stimulated lymphocytes
1977	Ofek et al.	Role of bacterial lectins in infection
1980	Pusztai	Interaction of <i>Phaseolus vulgaris</i> lectin with intestinal wall
1981	Reisner et al.	Use of lectins in bone marrow transplantation
1984	Yajko et al.	Combined use of lectin and enzyme in clinical identification of microorganisms
1987	Harban-Mendoza et al.	Control of root-knot nematodes by lectins
1988	De Oliveira et al.	Lectin and pancreas hypertrophy
1989	Diaz et al.	Root lectin as a specificity determinant in the <i>Rhizobium</i> -legume symbiosis
1990	Yamauchi & Minamikawa	Con A expression in <i>Escherichia coli</i> cells

Although some complex oligosaccharides are already known to specifically inhibit them, the classification of lectins is still done by their specificity for monosaccharides established by Makela (1957) who divided the lectin inhibitor

monosaccharides in four groups, depending on the configuration of the pyranosidic chain at C₃ and C₄: L-fucose (group I), galatose/N-acetyl-galactosamine (group II) and glucose/mannose (group III). Up to now, no lectin was

TABLE II
Chemical and Biological properties of some plant lectins

Species	Molecular Weight (KDal)		Molecular formula	Specificity		Metal	Carbohydrate
	Intact	Subunits		Cell	Carbohydrate		
<i>Abrus precatorius</i> (agg) ^{1,2}	126-135	A = 33; B = 36; B' = 37,5	A ₂ BB'	U	Gal	0	yes
<i>Adenia digitata</i> (modecin) ^{1,2}	57-63	A = 25-28; A = 31-35	AB	U	Gal	-	yes
<i>Adenia digitata</i> (modecin 6B) ^{1,2}	57	A = 27; B = 31	AB	U	Gal	-	yes
<i>Aleuria aurantiaca</i> ^{1,2}	72	A = 31	A ₂	U	L-Fuc	-	-
<i>Amphicarpa bracteata</i> ^{1,2}	135	A = 28,5; B = 36; G = 32	-	F	GalNAc-alfa-1,3-GalNAc	-	yes
<i>Arachis hypogaea</i> ^{1,2}	98-111	A = 25-28	A ₄	T	Gal-beta-1,3-GalNAc	Ca,Mn	-
<i>Artocarpus incisa</i> ³	43	A = 11; B = 15	A ₂ B ₂	U	-	-	yes
<i>Artocarpus integrifolia</i> ^{2,3}	43	A = 11; B = 15	A ₂ B ₂	T	Gal-beta-1,3-GalNAc	-	yes
<i>Bahunia purpurea</i> ^{1,2}	195	A = 44	A ₄	T, Tn	Gal-beta-1,3-GalNAc	0	yes
<i>Canavalia brasiliensis</i> ⁴	106*	A = 26	A ₄	U	Man > Glc	Ca,Mn	0
<i>Canavalia ensiformis</i> ^{1,2}	106	A = 26	A ₄	U	Man > Glc	Ca,Mn	0
<i>Canavalia gladiata</i> ⁵	106*	A = 26	A ₄	U	Man > Glc	Ca,Mn	0
<i>Canavalia maritima</i> ⁵	106*	A = 26	A ₄	U	Man > Glc	Ca,Mn	0
<i>Cratylia floribunda</i> ⁶	106*	A = 26	A ₄	U	Man > Glc	Ca,Mn	0
<i>Crotalaria juncea</i> ^{1,2}	120	A = 31	A ₄	U	Gal > GalNAc	Ca,Mn,Mg	yes
<i>Crotalaria striata</i> ⁷	-	A = 31	-	A ₁	GalNAc	-	-
<i>Cytissus sessilifolia</i> ^{1,2}	110	-	-	O(H)	GlcNAc > Fuc > Gal	-	-
<i>Datura stramonium</i> ^{1,2}	86	A = 40; B = 46	AB	U	GlcNAc (beta-1,4-GlcNAc)	0	-
<i>Dioclea grandiflora</i> ⁸	100	A = 26	A ₄	U	Man > Glc	Ca,Mn	0
<i>Dioclea guianensis</i> ⁹	100*	A = 26	A ₄	U	Man > Glc	Ca,Mn	0
<i>Dolichos biflorus</i> ^{1,2}	110-120	A = 27,3; B = 27,7	A ₂ B ₂	F	GalNAc-alfa-1,3-GalNAc	Ca,Mn,Mg,Zn	yes
<i>Erythrina cristagalli</i> ^{1,2}	56	A = 26; B = 28	AB	U	Gal-beta-1,4-GlcNAc	Mn,Ca	yes
<i>Erythrina indica</i> ^{1,2}	66-68	A = 30; B = 33	A ₂ ,AB,B ₂	U	GalNAc	Mn	yes
<i>Glycine max</i> ^{1,2}	120	A = 30	A ₄	A	GalNAc-alfa-1,3-Gal	Ca,Mn	yes
<i>Griffonia simplicifolia</i> A-4 ^{1,2}	114	A = 32	A ₄	A	GalNAc-alfa-1,3-Gal	Ca	yes
<i>Griffonia simplicifolia</i> B-4 ^{1,2}	114	B = 33	B ₄	B	Gal-alfa-1,3-Gal	Ca	yes
<i>Griffonia simplicifolia</i> IV ^{1,2}	56	A = 27; B = 29	AB	U	L-Fuc	-	yes
<i>Hura crepitans</i> ^{1,2}	120	A = 31	A ₄	U	GalNAc	-	yes
<i>Lathyrus cicera</i> ¹¹	49	A = 4,5; B = 20	A ₂ B ₂	U	Man > Glc	Ca,Mn	0
<i>Lathyrus ochrus</i> ¹¹	49	A = 4,5; B = 20	A ₂ B ₂	U	Man > Glc	Ca,Mn	0
<i>Lathyrus odoratus</i> ²	52	A = 5,8; B = 20	A ₂ B ₂	U	Man > Glc	Ca,Mn	0
<i>Lathyrus sativum</i> ^{1,2}	49	A = 4,4; B = 19	A ₂ B ₂	U	Man > Glc	Ca,Mn	0
<i>Lathyrus tingitanus</i> ^{1,2}	50	A = 5; B = 20	A ₂ B ₂	U	Man > Glc	Ca,Mn	0
<i>Lens culinaris</i> ^{1,2}	46	A = 5,7; B = 17,5	A ₂ B ₂	U	Man > Glc	Ca,Mn	0
<i>Lotus tetragonolobus</i> ^{1,2}	120	A = 27,4	A ₄	O(H)	L-Fuc	Ca,Mn	yes
<i>Maclura pomifera</i> ^{1,2}	40-46	A = 10; B = 12	A ₄ ,A ₃ B,A ₂ B ₂ ,AB ₃ ,B ₄	T,Tn	Gal-beta-1,3-GalNAc	0	0
<i>Macrotyloma axillare</i> ^{1,2}	108	A = 27; B = 27	A ₂ B ₂	A ₁	GalNAc	Ca,Mn,Mg,Zn	yes
<i>Momordica charantia</i> ^{1,2}	115-129	A = 27-29; B = 30-36	A ₂ B ₂	U	GalNAc	-	yes
<i>Onobrychis viciifolia</i> ^{1,2}	53	A = 26,5	A ₂	U	Glc > Man	Ca,Mn,Mg	yes
<i>Pisum sativum</i> ^{1,2}	50	A = 5,7; B = 17	A ₂ B ₂	U	Man > Glc	Ca,Mn	yes
<i>Phaseolus lunatus</i> ^{1,2}	(62) ₂₋₄	A = 31; A' = 31; B = 31	A ₄ ,A ₃ B,A ₂ B ₂ ,AB ₃ ,B ₄	A	GalNAc-alfa-1,3-Gal	Ca,Mn	yes
<i>Phaseolus vulgaris</i> ^{1,2}	126	A = 31; B = 31	A ₄ ,A ₃ B,A ₂ B ₂ ,AB ₃ ,B ₄	U	Gal-beta-1,4-GalNAc-beta-1,2,Man	Ca,Mn	-
<i>Vatairea macrocarpa</i> ¹⁰	-	A = 26	-	U	Gal	-	yes
<i>Vicia cracca</i> (Man) ^{1,2}	44	A = 5,8; B = 17,5	A ₂ B ₂	U	Man > Glc	Ca,Mn	yes
<i>Vicia cracca</i> (GalNAc) ^{1,2}	114	A = 33	A ₄	A	GalNAc-alfa-1,3-Gal	Ca,Mn	yes
<i>Vicia ervilia</i> ^{1,2}	53	A = 4,7; B = 21	A ₂ B ₂	U	Man > Glc	Ca,Mn	yes
<i>Vicia faba</i> ^{1,2}	52,5	A = 15,6; B = 20,7	A ₂ B ₂	U	Man > Glc	Ca,Mn	yes
<i>Vicia graminea</i> ^{1,2}	105	A = 26	A ₄	N	(Gal-1,3-GalNAc)clustered	Ca,Mn	yes
<i>Vicia sativa</i> ^{1,2}	40	A = 6; B = 14	A ₂ B ₂	U	Man	Ca,Mn	yes
<i>Vicia villosa</i> ^{1,2}	94-120	A = 33,6; B = 35,9	A ₄ ,A ₃ B,A ₂ B ₂ ,AB ₃ ,B ₄	A	GalNAc-alfa-1,3-Gal	Mn,Zn	yes
<i>Wistaria floribunda</i> (agg) ^{1,2}	(60) ₁₋₄	A = 28-32	A ₂₋₈	F,A	GalNAc-alfa-1,3-Gal	-	yes

* Calculated based on the subunit molecular weight; A, human blood group A; B, human blood group B; O(H) human blood group O(H); N, human blood group N; Forssman disaccharide; T, T antigen; Tn, Tn antigen; U, undefined specificity; Fuc, fucose; Gal, galactose; GalNAc, N-acetyl-galactosamine; Glc, glucose; GlcNAc, N-acetyl-glucosamine; Man, mannose; 1, Liener et al., 1986; 2, Wu et al., 1988; 3, Moreira & de Oliveira, 1983; 4, Moreira & Cavada, 1984; 5, Moreira et al., 1985; 6, De Oliveira et al., 1991; 7, De Oliveira et al., 1989; 8, Moreira et al., 1983; 9, Granjeiro et al., 1989; 10, Sales et al., 1989; 11, Rougé & Cavada, 1984.

found to react with sugars from Makela's group IV (idose, gulose, L-glucose and L-xylose). Nowadays, two new groups are used together with the Makela's groups: the N-acetyl-glucosamine and the sialic acid groups. Apparently, only in the groups of N-acetyl-galactosamine/galactose and L-fucose it was found known blood group specific lectins. This is not so surprisingly since the above sugars are involved, as determinants, in the structure of the A (N-acetyl-galactosamine), B (D-galactose) and O(H) (L-fucose) human blood antigens.

It is easier to find the appropriate affinity chromatography system once the sugar specificity is established. Thus, Sephadex (a commercially available polymer of dextran) can be used for isolation of glucose/mannose specific lectins

(Moreira et al., 1983; Moreira & Cavada, 1984; De Oliveira et al., 1991); epychlorohidrin treated gum guar (a galactose containing natural plant product) is used to isolate galactose specific lectins (Sales et al., 1989); oligosaccharides or glycoproteins as fetuin (Pinto, 1987); and blood group A₁-substance (De Oliveira et al., 1989). Synthetic derivatives of galactosides are also used for this purpose (Lis & Sharon, 1981).

The lectins are, usually, glycoproteins with variable sugar contents, with some exceptions as the well studied con A (Goldstein & Poretz, 1986) and the lectins from *Canavalia brasiliensis* (Moreira & Cavada, 1984), *Dioclea grandiflora* (Moreira et al., 1983), *Dioclea guianensis* (Vasconcelos et al., 1990), *Cratylia flori-*

bunda (De Oliveira et al., 1991), and need divalent cations to display full activity. The molecular weights of plant lectins are also variable. In general, they are oligomers and the tetrameric structure is the most common as in the lectins of *Canavalia ensiformis* (Goldstein & Poretz, 1986) and *Dioclea grandiflora* (Moreira et al., 1983), both made of identical subunits; the lectins from *Lathyrus ochrus* (Rougé & Cavada, 1984) and *Ricinus communis* (Nicholson et al., 1974) with two different subunits arranged in the A₂B₂ form; and the *Phaseolus vulgaris* lectins, a family of five isolectins, E₄, E₃L, E₂L₂, EL₃, L₄ (Pusztai & Stewart, 1978) (Table II).

Some lectins show a pattern of broken subunits, apparently characteristic of those from plants belonging to the tribe Diocleae. The main cleavage point both in *Canavalia ensiformis* (Wang et al., 1971) and *Dioclea grandiflora* (Richardson et al., 1984; Ainouz et al., 1987) lectins, for instance, is between the amino acids Asn (118) and Ser (119) giving two almost simetrical subunits from the 237 amino acid chain. Other lectins from the same tribe, which did not have their primary structure yet determined, show similar fragments by polyacrylamide gel electrophoresis (PAGE-SDS) such as *Canavalia brasiliensis*, *Cratylia floribunda*, *Dioclea sclerocarpa* (Moreira et al., 1985) and *Dioclea guianensis* (Vasconcelos et al., 1991).

Due to their peculiar properties, the lectins are used as a tool, both for analytical and preparative purposes in biochemistry, cellular biology, immunology and related areas. In agriculture and medicine, the use of lectins greatly improved in the last few years (Table III).

The lectins are found in almost all edible plants and exposure of men and animals to them is inevitable. Experimentally it has been shown that some lectins are very resistant to the gut enzymes and found to be still active in the faeces of rats or human beings when they have been fed with food sources that contain these proteins (Brady et al., 1978; Pusztai, 1980; Higuchi et al., 1983; Nakata & Kimura, 1985; Kilpatrick et al., 1985; Liener, 1986; Vasconcelos et al., 1989). This fact is of utmost nutritional importance, because the lectin-derived aminoacids are non available for the animals and the intact or partially digested

lectin can bind to the epithelial cells lining the intestines (Etzler & Branstrator, 1974; King et al., 1980).

TABLE III

Major applications of lectins

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1. Isolation, purification and structural studies of glycoconjugates.
 2. Studies of cellular and subcellular membrane components.
 3. Studies of virus surface components.
 4. Studies of changes in cell surfaces upon malignant transformation.
 5. Mitogenic stimulation of lymphocytes and studies of cell division, chromosomal constitution of cells and chromosomal abnormalities.
 6. Cell separation.
 7. Diagnosis and identification of microorganisms.
 8. Blood typing.
 9. Drug carriers.
 10. Plant defense against predators.
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Jaffé (1960) was the first to attribute the poor performance of rats that follows the ingestion of raw kidney beans (*Phaseolus vulgaris*) to the interaction of the lectin with receptors on the surface of the intestinal cells. Nowadays it is clear that this lectin, after interaction with the intestine, is endocytosed and cause many disturbances at systemic level. Thus, an enlargement of the intestine, liver and pancreas was observed when the pure *Phaseolus vulgaris* lectin was fed to rats (De Oliveira et al., 1988). Apparently, this enlargement in the pancreas may be responsible for the observed decrease in the insulin levels of the rats (De Oliveira, 1986; Pusztai et al., 1986). Additionally the kidney bean lectin fed rats had thymus atrophy (Green, 1984; De Oliveira, 1986). This atrophy may, perhaps, be related to the unusual bacterial proliferation in the gut since the immunological system may have been depressed (Jayne-Williams & Hewitt, 1972; Jayne-Williams & Burgess, 1974; Banwell et al., 1985). The ingestion of the *Phaseolus vulgaris* lectin also disturbs the intermediary metabolism with an increase in nitrogen excretion and in lipid catabolism (De Oliveira, 1986) leading to a general debility with the experimental animals showing lost of weight, inadequate development and eventually death.

The possible physiological functions of the plant lectins are still unclear. Their ultrastruc-

tural location and behavior during the plant life cycle are of extreme importance for the evaluation of any hypothesis on their functions.

The great majority of plant lectins are present in seed cotyledons where they are found in the cytoplasm (Mialonier et al., 1973) or in the protein bodies (Youle & Huang, 1976; Boisseau et al., 1984) and may constitute up to 10% of total nitrogen (Etzler, 1986).

Several hypotheses on the physiological functions of plant lectins were suggested, based on their general properties and location. Thus, lectins could play an important role as reserve proteins, as defense mechanism against pathogens (Mirelman et al., 1975; Barkai-Golan et al., 1978; Etzler, 1986) and in the plant-microorganism symbiosis (Hamblin & Kent, 1973; Bohlool & Schmidt, 1974; Dazzo and Truchet, 1983). Despite of the profusion of the literature dealing with the question of whether lectins are involved in determining specificity of *Rhizobium*-legume symbiosis, although circumstantial and suggestive evidences has been accumulated, only recently a clear cut evidence has been put forward by Diaz et al. (1989), who showed a change in specificity toward a given strain of *Rhizobium leguminosarum* by transgenic plants.

Plant lectins, apparently, participate in cell organization, embryo morphogenesis, phagocytosis and cell protection. In addition, an active role in both carbohydrate transport and fixation in the plant, as well as, in cell wall growth and elongation, in callus and protoplast induced mitosis and in pollen recognition is suggested (Knox et al., 1976).

The main characteristic of plant lectins is their sugar specific binding sites. It is evident that this property cannot be casual and has to be relevant for the plant physiology. Therefore, it must exist carbohydrate-containing receptors in the tissues where they are found or with which they may eventually be in contact. The identification, without ambiguity, of these receptors is an essential step for the acceptance of the hypothesis. Indeed, such receptors have been already described in seeds of *Vicia cracca* (Renkonem, 1960), *Pisum sativum*, *Canavalia ensiformis*, *Vicia faba*, *Vicia sativa* and *Ricinus communis* (Gansera et al., 1979; Gebauer et al., 1979). The potential role and significance

of these receptors, usually present in very small amounts, are not clear yet.

In *Dioclea grandiflora* and *Dioclea sclerocarpa* (Horta-Barros et al., 1987) these receptors were found in a large amount and it is even possible to prepare an affinity column with them, for isolating lectins.

With respect to the metabolism of lectins during the plant life cycle, the earliest report was done by von Eysler & von Portheim in 1911 and Schneider in 1912. They showed that the disappearance of the lectin in the nutrient reserve organs, appears to be similar to the overall rate of protein degradation (Kocourek, 1986). Later, similar behavior were found for the lectins from *Lens culinaris* (Howard et al., 1972;), *Phaseolus vulgaris* (Mialonier et al., 1973), *Vicia sativa* (Gracis & Rougé, 1977), *Pisum sativum* (Rougé 1976), and three species of *Lathyrus* (Rougé & Pere, 1982). Differently, the degradation of the *Ricinus communis* lectin is slower than that of the other proteins (Youle & Huang, 1976).

When the presence of *Canavalia brasiliensis* lectins was examined during seed germination and early stages of plantlet development, by hemagglutinating activity, PAGE-SDS electrophoresis, Sephadex G-50 affinity chromatography and immunochemistry, both in dark and the presence of the light, a delayed disappearance was also found. An increase of hemagglutinating activity was observed in the early stages of germination, probably due to the disappearance of a lectin receptor (or inhibitor) which is apparently extracted with the lectin. However, this increase in activity was not in parallel with the amount of lectin measured either by affinity chromatography or by PAGE-SDS electrophoresis and immunochemical methods. The characteristic lectin bands can be detected until the time of abscission, when the cotyledons are almost completely exhausted and no reserve protein bands are detected (Moreira & Cavada, 1984).

During seed development, the lectin synthesis and accumulation showed a slight time delay, when compared with the bulk of reserve protein. In the early stages of seed development, the lectin was synthesized as a precursor, with a poor hemagglutinating activity but with high affinity for dextran. This precursor was shown to have lower pI and a molecular weight slightly

higher than the fully active lectin, with and extra tetrapeptide in the N-terminal portion. This precursor is then processed into the fully active lectin. In the final stages, on the other hand, the hemagglutinating activity decreases with no parallelism with the lectin amount as measured by affinity chromatography. This decrease in activity may be due to the presence of a newly synthesized receptor, or inhibitor (Silva, 1986).

These results clearly showed the difference in the processing of the *Canavalia brasiliensis* lectin during seed development and degradation, when compared with the bulk of reserve proteins. It was suggested a relevant role, although yet unknown, for the lectin in the physiology of the plant.

REFERENCES

- AGRAWAL, B. B. L. & GOLDSTEIN, I. J., 1965. Specific binding of concanavalin A to cross linked dextran gels. *Biochem J.*, 96: 230.
- AINOUZ, I. L.; MOREIRA, R. A.; CAMPOS, F. A. P.; RICHARDSON, M.; BEGBIE, R.; STEWART, J. C.; WATT, W. B. & PUSZTAI, A., 1987. The isolation and amino acid sequence of the beta and gamma subunits of the lectin from seeds of *Dioclea grandiflora*. *Phytochem.*, 26: 1435-1440.
- BANWELL, J. G.; HOWARD, R.; COOPER, D. & COSTERTON, J. W., 1985. Intestinal microbial flora after feeding phytohemagglutinin lectins. *Appl. Envir. Microbiol.*, 50: 68-80.
- BARKAI-GOLAN, R.; MIRELMAN, D. & SHARON, N., 1978. Studies on growth inhibition by lectins of *Penicilia* and *Aspergilli*. *Arch. Microbiol.*, 116: 119-124.
- BOHLOOL, B. B. & SCHMIDT, E. L., 1974. Lectins: a possible basis for specificity in the *Rhizobium*-legume root nodule symbiosis. *Science*, 185: 269-271.
- BOISSEAU, C.; CAUSSE, H.; MOISAND, A.; PERE, D.; CAVADA, B. S. & ROUGE, P., 1984. Localization and biosynthesis of *Lathyrus ochrus* seed lectin, p. 651-654. In P. Arnaud; J. Bienvenu & P. Laurent, (Eds) *Marker protein in Inflammation*, vol 2, Walter de Gruyter, Berlin.
- BRADY, P. G.; VANNIER, A. M. & BANWELL, J. G., 1978. Identification of the dietary lectin, wheat germ agglutinin, in human intestinal contents. *Gastrointerol.*, 75: 236-239.
- DAZZO, F. B. & TRUCHET, G. L., 1983. Interactions of lectins and their saccharide receptors in the *Rhizobium*-legume symbiosis. *J. Membr. Biol.*, 73: 1-16.
- DE OLIVEIRA, J. T. A., 1986. *Lectins. The effects of dietary Phaseolus vulgaris lectins on the general metabolism of monogastric animals*. PhD thesis, University of Aberdeen, UK.
- DE OLIVEIRA, J. T. A.; PUSZTAI, A. & GRANT, G., 1988. Changes in organs and tissues induced by feeding of purified kidney bean (*Phaseolus vulgaris*) lectins. *Nutr. Res.*, 8: 943.
- DE OLIVEIRA, J. T. A.; RAMOS, M. V.; MOREIRA, R. A., & CAVADA, B. S., 1989. An anti-blood group A₁ lectin from *Crotalaria striata* seeds. *Arq. Biol. Tecnol.*, 32: 152.
- DE OLIVEIRA, J. T. A.; CAVADA, B. S. & MOREIRA, R. A., 1991. Isolation and partial characterization of a lectin from *Cratylia floribunda* Mart. seeds. *Rev. Brasil. Botânica*, 14: 63-68.
- DIAZ, C. L.; MELCHERS, L. S.; HOOYKAAS, P. J. J.; LUGTENBERG, B. J. J. & KIJNE, J. W., 1989. Root lectin as a determinant of host-plant specificity in *Rhizobium*-legume symbiosis. *Nature*, 338: 579-581.
- ETZLER, M. E., 1985. Plant lectins: Molecular and Biological aspects. *Annu. Rev. Plant Physiol.*, 36: 209-234.
- ETZLER, M. E., 1986. Distribution and function of plant lectins. In I. E. Liener; N. Sharon & I. J. Goldstein, (Eds). *The lectins*, Academic Press, Orlando.
- ETZLER, M. E. & BRANSTRATOR, M. L., 1974. Differential localization of cell surface and secretory components in rat intestinal epithelium by use of lectins. *J. Cell. Biol.*, 62: 329.
- GANSERA, R.; SCHURZ, H. & RUDIGER, H., 1979. Lectin associated proteins from the seeds of Leguminosae. *Hoppe-Seyler's Z. Physiol. Chem.*, 360: 1579-1585.
- GEBAUER, G.; SCHLITZ, E.; SCHIMPL, A. & RUDIGER, H., 1979. Purification and characterization of a mitogenic lectin and lectin binding protein from *Vicia sativa*. *Hope-Seyle's Z. Physiol. Chem.*, 360: 1727.
- GOLD, J. & BALDING, P., 1975. *Receptor-specific proteins. Plant and animal lectins*. Excerpta Medica, Amsterdam.
- GOLDSTEIN, I. J. & HAYES, C. E., 1978. The lectins: carbohydrate binding proteins of plant and animals. *Adv. Carbohydr. Chem. Biochem.*, 35: 127-340.
- GOLDSTEIN, I. J. & PORITZ, R. D., 1986. Isolation and properties of lectins. In I. E. Liener; N. Sharon & I. J. Goldstein, (Eds). *The lectins: properties, functions and applications in biology and medicine*. Academic Press, London.
- GOLDSTEIN, I. J.; HUGHES, R. C.; MONSIGNY, M.; OZAWA, T. & SHARON, N., 1980. What should be called a lectin? *Nature*, 285: 66.
- GRACIS, J. P. & ROUGÉ, P., 1977. *Bull. Soc. Bot. Fr.* 124: 301-306.
- GRANJEIRO, T. B.; VASCONCELOS, I. M.; CAVADA, B. S.; MOREIRA, R. A. & DE OLIVEIRA, J. T. A., 1989. Lectins from *Dioclea lasiophylla* Duke. Studies on extraction, sugar and erythrocyte specificity. *Cienc. Cult.*, 415: 807.
- GREEN, F., 1984. *Local (intestinal and systemic) responses of animals to ingested Phaseolus vulgaris lectins: mechanisms of lectin toxicity*. PhD Thesis, University of Aberdeen, U. K.
- HAMBLIN, J. & KENT, S. P., 1973. Possible role of phytohemagglutinin in *Phaseolus vulgaris* L. *Nature*, 245: 28.
- HIGUCHI, M.; SUGA, M. & IWAI, K., 1983. Participation of lectin in biological effects of raw winged bean seeds on rats. *Agric. Biol. Chem.*, 47: 1879.
- HORTA-BARROS, A. C.; CAVADA, B. S.; OLIVEIRA, J. T. A.; CRISÓSTOMO-PINTO, F. S.; AL-

- MEIDA-SILVA, L. M. & MOREIRA, R. A., 1987. Receptores endógenos de lectinas de sementes da tribo *Diocleae*. XI Reunião Nordestina de Botânica, Fortaleza (CE), Abstracts.
- HOWARD, I. K.; SAGE, H. J. & HORTN, C. B., 1972. *Arch. Biochem. Biophys.*, 149: 323-3267, quoted by Etzler, M. E. (1986).
- JAFFÉ, W. C., 1960. *Arzmeim-Forsch.*, 10: 1012-1016, quoted by Liener (1986).
- JAFFÉ, W. C., 1969. Hemagglutinins. In I. E. Liener *Toxic constituents of Plant Food-stuff*. Academic Press, New York.
- JAYNE-WILLIAMS, D. J. & BURGERS, C. D., 1974. Further observations on the toxicity of navy beans (*Phaseolus vulgaris*) for japanese quail (*Coturnix coturnix japonica*). *J. Appl. Bacteriol.*, 37: 149-169.
- JAYNE-WILLIAMS, D. J. & HEWITT, D., 1972. The relationship between the intestinal microflora and the effects of diets containing raw navy beans (*Phaseolus vulgaris*) on the growth of Japanese quail (*Coturnix coturnix japonica*). *J. Appl. Bacteriol.*, 35: 331-345.
- KILPATRICK, D. C.; PUSZTAI, A.; GRANT, G.; GRAHAN, C. & EWEN, S. W. B., 1985. Tomato lectin resists digestion in mammalian alimentary canal and binds to intestinal villi without deleterious effects. *FEBS Letters*, 185: 299.
- KING, T. P.; PUSZTAI, A. & CLARKE, E. M. W., 1980. Immunocytochemical localization of ingested kidney bean (*Phaseolus vulgaris*) lectins in rat gut. *Histochem. J.*, 12: 201-208.
- KNOX, R. B.; CLARKE, A.; HARRISON, S.; SMITH, P. & MARCHALONIS, J. J., 1976. Cell recognition in plant: determinants on the stigma surface and their pollen interactions. *Proc. Natl. Acad. Sci. USA*, 73: 2788.
- KOCOUREK, J., 1986. Historical Background. In I. J. Liener; N. Sharon, & I. J. Goldstein (eds). *The Lectins. Properties, Functions and Applications in Biology and Medicine*. London, Academic Press.
- KOCOUREK, J. & HOREJSI, V., 1983. A note of the recent discussion of definition of the term "lectin". In T. C. Bog-Hansen, & G. A. Spengler, (Eds). *Lectins: Biology, Biochemistry, Clinical Biochemistry*. Proceedings of the 5th Lectin Meeting. Vol 3, Walter de Gruyter Berlin-New York.
- LIENER, I. E., 1986. Nutritional significance of lectins in the diet. In I. E. Liener; N. Sharon & I. J. Goldstein, (Eds). *The Lectins: Properties, Functions, and Applications in Biology and Medicine*. Academic Press, New York.
- LIENER, I. E.; SHARON, N. & GOLDSTEIN, I. J., 1986. *The Lectins: Properties, Functions, and Applications in Biology and Medicine*. Academic Press, New York.
- LIS, H. & SHARON, N., 1981. Lectins in higher plants. 371-447. In A. Marcus. *The Biochemistry of Plants*. Academic Press, vol 6, New York.
- MAKELA, O., 1957. Studies in hemagglutinins of *Leguminosae* seeds. *Ann. Med. Exp. Biol. Fenn.* 35 S11, 1-156.
- MARBAN-MENDOZA, N.; JEYAPRAKASH, A.; JANSSON, H. B.; DAMON JR R. A. & ZUCKERMAN, B. M., 1987. Control of root-knot nematodes on tomato by lectins. *J. Nematol.*, 19: 331-335.
- MIALONIER, G.; PRIVAT, J. P.; MONSIGNY, M.; KOHLEN, G. & DURAND, R., 1973. Isolement, propriétés physico-chimiques et localisation *in vivo* d'une phytohemagglutinine (lectine) de *Phaseolus vulgaris* L. (var. rouge). *Physiol. Veg.*, 11: 519-537.
- MIRELMAN, D.; GALUN, E.; SHARON, N. & LOTAN, R., 1975. Inhibition of fungal growth by wheat germ agglutinin. *Nature*, 256: 414-416.
- MOREIRA, R. A. & CAVADA, B. S., 1984. Lectin from *Canavalia brasiliensis* Mart. Isolation, characterization and behavior during germination. *Biol. Plant.*, 26: 113-120.
- MOREIRA, R. A. & DE OLIVEIRA, J. T. A., 1983. Lectins from the genus *Artocarpus*. *Biol. Plant.*, 25: 343-348.
- MOREIRA, R. A.; BARROS, A. C. H.; STEWART, J. C. & PUSZTAI, A., 1983. Isolation and characterization of a lectin from the seeds of *Dioclea grandiflora* Mart. *Planta*, 158: 63-69.
- MOREIRA, R. A.; BARROS, A. C. H.; OLIVEIRA, J. T. A. & RICHARDSON, M., 1985. Comparative studies of lectins from seeds of the tribe *Diocleae*. *Arq. Biol. Tecnol.*, 28: 173.
- NAKATA, S. & KIMURA, T., 1985. Effect of ingested toxic bean lectins on the gastrointestinal tract in the rat. *J. Nutr.*, 115: 1621-1629.
- NICHOLSON, G. L.; BLAUSTEIN, J. & ETZLER, M. E., 1974. Characterization of two plant lectins from *Ricinus communis* and their quantitative interaction with a murine lymphoma. *Biochemistry*, 13: 196.
- PINTO, F. S. C., 1987. *Isolamento e caracterização das lectinas de sementes de Artocarpus incisa. L. var. seminiifera*. MSc. Thesis. University of Ceará. Brazil.
- PUSZTAI, A., 1980. Nutritional toxicity of the kidney bean (*Phaseolus vulgaris*) *Rep. Rowett Inst.*, 36: 110.
- PUSZTAI, A., 1989. Lectins. In P. R. Cheeke, *Toxicants of Plant Origin*, vol III. *Proteins and Amino Acids*, CRC Press, Boca Raton, USA.
- PUSZTAI, A. & STEWART, J. C., 1978. Isolectins of *Phaseolus vulgaris*. *Physicochemical studies. Biochim. Biophys. Acta*, 536: 38.
- PUSZTAI, A.; CROY, R. R. D.; GRANT, G. & STEWART, J. 1983. Seed lectins: distribution, location and biochemical role. In J. Daussant; J. Mosse, & J. Vaugham, (Eds). *Seed Proteins*, Academic Press, New York.
- PUSZTAI, A.; GRANT, G. & DE OLIVEIRA, J. T. A., 1986. Local (gut) and systemic responses to dietary lectins. *IRCS Med. Sci.*, 14: 205.
- RENKONEN, H. O., 1960. The development of hemagglutinins in the seeds of *Vicia cracca*. *Ann. Med. Exper. Biol. Fenniae*, 38: 26.
- RICHARDSON, M.; CAMPOS, F. A. P.; MOREIRA, R. A.; AINOUS, I. L.; BEGBIE, R.; WATT, W. B. & PUSZTAI, A., 1984. The complete amino acid sequence of the major alfa-subunit of the lectin from the seeds of *Dioclea grandiflora* Mart. *Europ. J. Biochem.*, 114: 101-111.
- ROUGÉ, P., 1976. Biosynthese des hemagglutinines au cours de la maturation des graines de pois. *C. R. Acad. Sci. Paris*, 282: 621-623.
- ROUGÉ, P. & CAVADA, B. S., 1984. Isolation and partial characterization of two isolectins from *Lathyrus ochus* L. (DC). *Plant Science Letters*, 32: 21-27.

- ROUGÉ, P. & PÉRE, D., 1982. Occurrence of lectin during the cycle of *Lathyrus* species. 137-150. In T. C. Bog-Hansen, *Lectins: Biology, Biochemistry, Clinical Biochemistry*. Proceedings of the 5th Lectin Meeting, Walter de Gruyter, Berlin-New York, vol II.
- SALES, P. V. P.; DE OLIVEIRA, J. T. A.; MOREIRA, R. A. & CAVADA, B. S., 1989. Isolation and characterization of a lectin from *Vatairea macrocarpa* Duke seeds. *Arq. Biol. Tecnol.*, 32: 151.
- SHARON, N. & LIS, H., 1989. *Lectins*, Chapman and Hall, London.
- SILVA, L. M. A., 1986. *Lectinas de Canavalia brasiliensis*. *Comportamento durante a maturação da semente*. MSc Thesis, University of Ceará. Brazil.
- STILLMARK, H., 1888. *Über Ricin, ein giftiges Ferment aus den Samen von Ricinus comm. L. und einigen anderen Euphorbiaceen*. Thesis Univ. Dorpat, quoted by Kocourek 1986.
- TOMS, G. C. & WESTERN, A., 1971. Phytohemagglutinins. In J. B. Harbone; D. Boulter & B. L. Turner (eds). *Chemotaxonomy of Leguminosae*. Academic Press, London.
- VASCONCELOS, I. M.; FIRMINO, F.; MOREIRA, R. A.; CAVADA, B. S.; GUIMARÃES, F. A. & DE OLIVEIRA, J. T. A., 1989. Nutritional studies of *Canavalia brasiliensis* Mart. seeds. *Cien. Cult.*, 41S: 786.
- VASCONCELOS, I. M.; CAVADA, B. S.; MOREIRA, R. A. & DE OLIVEIRA, J. T. A., 1991. Purification and partial characterization of a lectin from the seeds of *Dioclea guianensis*. *J. Food Biochem.*, 15: 137-154.
- WANG, J. L.; CUNNINGHAM, B. A. & EDELMAN, G. M., 1971. Unusual fragments in the subunit structure of Concanavalin A. *Proc. Natl. Acad. Sci. USA*, 68: 1130-1134.
- WU, A. M.; SUGII, S. & HERP, A., 1988. A guide for carbohydrate specificities of lectins. In A. M. Wu, *The Molecular Immunology of Complex Carbohydrates*. Plenum Publishing Co. New York.
- YAJKO, D. M.; CHU, A. & HADLEY, W. K., 1984. Rapid confirmatory identification of *Neisseria gonorrhoeae* with lectins and chromogenic substrates. *J. Clin. Microbiol.*, 19: 380-382.
- YAMAUCHI, D. & MINAMIKAWA, T., 1990. Structure of the gene encoding convalanin A from *Canavalia gladiata* and its expression in *Escherichia coli*. *FEBS Letters*, 260: 127-130.
- YOULE, R. J. & HUANG, A. H. C., 1976. Protein bodies from the endosperm of castor bean subfraction, protein components, lectins and changes during germination. *Plant Physiol.*, 58: 703-709.