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PLANT LECTINS WITH SPECIAL REFERENCE TO ORCHIDS — A BRIEF REVIEW

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Abstract

A new era of research is being devoted to decipher different roles of lectins, the carbohydrate-binding molecules of plant. Studies carried out in the last two decades demonstrate the specific capacity of lectins to recognize diverse sugar structures and mediate a variety of biological processes such as cell-cell and host– pathogen interactions, serum-glycoprotein turnover and innate immune responses. They also provide a basis for a unique mode of classification of proteins in much wider aspects. Furthermore, the chemistry and biology of these proteins have been responsible for providing greater insight into the understanding of their functions and their role in biological system. The extensive source of information necessitates a simultaneous analysis of all probable members of this group of proteins to develop a broader perspective of the functionalities as well as potential uses of these lectins with special reference to Orchids. This paper is an attempt in this direction.

Introduction

LECTINS ARE a class of widely occurring proteins present in almost all plant species including lower forms like mosses and fungi. They include a heterogeneous group of proteins having varied biological roles. The study of lectins, known as Lectinology, dates back to 1888, when extracts from castor beans were found to agglutinate red blood cells (Stillmark, 1888). Since then hundreds of lectins have been isolated from plants (both monocotyledonous and dicotyledonous), and also from animals (Park and Kim, 1987; Waard *et al.*, 1976). The present article attempts to review briefly the structure and functions of lectins with special reference to the ones isolated from orchids.

Since the early work on legume lectins by Renkonnen (1948), and Boyd and Reguera (1949), momentum on lectin research literally gained pace in 1980s. With the progress in the purification and characterization of lectins, efforts are being made to propose a unifying definition to include this large group of heterogeneous plant proteins. According to Goldstein *et al.*(1980), lectins are carbohydrate binding proteins of non-immune origin, which agglutinate cells or precipitate glycoconjugates. Goldstein and Poretz (1986), on the basis of carbohydrate-binding specificity, classified plant lectins into five groups *i.e* glucose/mannose-specific, galactose/N-acetylgalactosamine-specific, N-acetylglucosamine – specific, and sialic acid specific. Van Damme *et al.* (1998) categorized lectins into four major types *i.e.* merolectins, hololectins, chimerolectins, and superlectins on the basis of the overall structure of their subunits.The richest source of plant lectins are the seeds, or more generally the storage organs of plants. For most plants studied so far, lectins have been isolated from seeds, but roots, tubers, bulbs, bark or leaves also serve as starting materials for the isolation of lectins (Rudiger, 1998).

The most popular method for detecting the presence of lectin is to test the plant extracts for their ability to agglutinate human or other mammalian erythrocytes. This classical method, used a century ago by Mitchell (1860) and Stillmark (1888), is still favoured for its simplicity and accuracy in spite of few minor discrepancies.The characterization of lectins can be performed by the standard protocols for protein purification, which include affinity, ion-exchange, size-exclusion, and hydrophobic chromatography. At present, the most preferred technique employed for purification of lectin is affinity chromatography done on immobilized carbohydrates or glycoproteins (Dhuna *et al.*, 2005; Echemendia – Blanco *et al.*, 2009; Park and Kim, 1987; Smeets *et al.*, 1997; Sudmoon *et al.*, 2008; Waard *et al.*, 1976).

Increased use of lectins in chemical and biological research has prompted the development of many methods for their purification. Because of its specificity, affinity chromatography has been most widely used and several types of ligands and support media have been employed. Agrawal and Goldstein (1965) first employed the technique of affinity chromatography for the purification of Concanavalin A. (Datta and Basu,1983) prepared extracts from the seeds of *Erythrina variegata* by ammonium sulfate precipitation method and then purified the lectin fraction by affinity chromatography on acid treated Sepharose 4B with a yield of 81%.

The first lectin to be isolated from an orchid is LOA (*Listera ovata* Agglutinin) from the leaves of *Listera ovata* (Van Damme *et al.*, 1987). The lectin has been purified

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by affinity chromatography using an immobilized mannose column. Since then, lectins are isolated and purified from different plant and animal species using specific columns of affinity chromatography. The columns prepared include different sugars as well as glycoproteins.

Plant lectins inhibit HIV replication in lymphocyte cell cultures through inhibition of virus/ cell fusion (Hammar *et al.*, 1989; Matsui *et al.*, 1990). It has been found that among the variety of sugar- binding lectins, Manose binding lectins (MBLs) are the most potent inhibitor of HIV replication in cell culture (Balzarini *et al.*,1991, 1992). These lectins prevent fusion of HIV particles with their target cells. Hajto *et al.* (2005) have isolated a lectin component of *Viscum album* (VAA) and reported that VAA-1 has notable immunomodulatory effect when injected in a murine model. The total thymocyte cell count in the thymus increased significantly indicating an increase in $CD8^+$ thymocyte population whereas $CD4^+$cells do not increase in number.

Role of Plant Lectins

Lectins in Plant Defense

Lectins have been implicated in plant defense against pathogens and pests, and a host of evidence is available to substantiate this claim. Most of the lectins are thermostable and remain active over a wide range of pH. Sequeira and Graham (1997) immobilized avirulent strains of *Pseudomonas solanacearum in vitro* using potato lectin.

Insecticidal Activities of Plant Lectins

The insecticidal activities of lectins were demonstrated based on the manner in which they bind to the glycoprotein receptors abundantly present on the luminal surface of the gut epithelium of phytophagous insects.

Toxicity of Plant Lectins

Plant lectins can be toxic to higher animals and even to the human beings. Most of the present knowledge about the toxicity of plant lectins on animals and humans has come from feeding experiments with purified Phytohemagglutinin (PHA) and accidental poisoning of humans by raw or insufficiently cooked beans. The reasons for this toxicity have been attributed to the fact that, when lectins enter the brush border cells of the intestine, they induce an enhanced metabolic activity that eventually leads to hyperplasia and hypertrophy of the small intestine (Pusztai *et al.*, 1990).

Lectins in Symbiotic Relationship

Lectins have effectively been implicated in *Rhizobium* – plant root nodule interaction (Bohlool and Schmidt, 1974; Chandrika and Shaila, 1987; Diaz *et al.*, 1989; Komath *et al.*, 2006). When specific binding takes place, lectins from both bacteria and plant roots can evoke physiological and biochemical responses (Diaz *et al.*, 1989; Lis and Sharon, 1998; Rudiger and Gabius, 2001). It is also apparent that lectins indulge in the production of lipochitooligosaccharides or nodulation factors by the bacteria (Kalsi and Etzler, 2000). These findings actually open up a very interesting area in orchid lectinology to find out the role of lectins in building the symbiotic relationship between orchid roots and mycorrhizae if any.

Cytotoxic Effects of Lectin

Cytotoxic effects of plant lectins on HIV infections and cancer cell lines have been a focus of study by a number of workers. Several reports emphasized the fact that mannose- specific lectins interfere with HIV gp 120, a viral envelope glycoprotein and subsequently in its mode of infection (Balzarini *et al.*,1991; Hammar *et al.*, 1989; Lifson *et al.*,1986; Muller *et al.*,1988; Robinson *et al.*, 1987). Muller *et al.*,(1988) reported that D-mannose binding lectin from *Gerardia savaglia* inhibits HIV-1 infection of H-9 cells. MBLs from *Galanthus nivalis* (GNA), *Hisppeastrum* hybrid (HHA), *Narcissus pseudonarcissus* (NPA), and *Listera ovata* (LOA) have inhibitory effects on HIV (both I and II) induced cytopathogenicity in MT-4 cells and syncytium formation between HIV infected HUT–78 cells and MOLT-4 cells (Balzarini *et al.*, 1991).

The anti-viral activity of lectins has stimulated investigations on the contribution of lectins in cancer research and therapy. According to Gabius (1987), alterations in malignant cells as well as reduction in cancer cell tumorigenicity can be detected by some lectins. A lectin from *Agaricus bisporus* is found to reversibly inhibit the proliferation of colonic cancer cell lines (Parslew *et al.*, 1999). Dhuna *et al.* (1995) have shown that lectin from *Arisaema tortuosum* inhibits *in vitro* proliferation of human cancer cell lines *i.e.* HT 29, SiHa and OVCAR – 5. Another monocot lectin, isolated and purified from *Typhonium divaricatum* (Araecaceae), is shown to have anti-viral activities against HSV-II and anti-proliferative effect on various human cancer cell lines like Pro-01 (prostate), Lm-04 (lung), Bre-04 (breast), Hep G2 (liver), and HeLa (cervix). Lia *et al.*, (2008) have observed anti-tumor activity of a lectin isolated and purified from *Polygonatum cyrtonema* (PCL) as it executes a dose-dependent growth inhibition of HeLa cells. Based on the morphological studies, it is suggested that PCL induces HeLa cell apoptosis.

Lectins from Orchidaceae

This review clearly establishes that lectins are widely occurring plant proteins with ample versatility in their activities. However, most of the studies are carried out on lectins isolated from non-orchid plants. A recent estimate shows that only 6 species of the family Orchidaceae have been exploited for the isolation of lectins thus accounting only 1.75% of all the angiosperm plants (Gabius *et al.*, 2004). This has entailed the present authors to lay emphasis on the orchid lectins. Many monocot mannose- binding lectins have been observed to have anti-viral and anti-tumor activity on a number of human cancer lines. The first orchid lectin to be isolated was a mannose binding lectin from the leaves of *Listera ovata* (Van Damme *et al.*, 1987) and was named as *Listera ovata* Agglutinin (LOA). Since then lectins have been isolated from a few orchid species *i.e. Cymbidium* hybrid, *Dendrobium lindleyanum,* and *Epipactis helleborine*. Orchid lectins are mostly involved in plant defense. However, LOA has been found to be highly inhibitory to HIV 1 and 2 in MT-4 cells. It also exhibits marked anti-viral activity against cytomegalovirus and influenza virus-A in Hela cells *in vitro* (Balzarini *et al.*, 1992). Similar anti-viral activities have also been shown by *Cymbidium* hybrid Agglutinin (CHA) and *Epipactis helleborine* Agglutinin (EHA) (Balzarini *et al.*, 1992). It is therefore, apparent from the earlier studies that orchid lectins have tremendous medicinal significance.

Lectin from Cymbidium Hybrid (Cha)

A mannose specific lectin has been isolated from the leaves of *Cymbidium* hybrid using affinity chromatography on immobilized mannose. The lectin is a dimer (25kDa) composed of 12.5kDa subunits. It is not glycosylated and occurs as a mixture of isolectins (Van Damme *et al.*, 1994). The Cha has been found to agglutinate only rabbit erythrocytes but not human RBC. Further, the lectin is highly inhibitory to HIV-1 and HIV-2 at concentrations of 0.08 and 0.06 μg ml l^{-1} respectively (Balzarini *et al.*, 1992). No further information is available on its applications and availability.

Lectin from Epipactis helleborine (Eha)

At present two different lectins have been isolated from *Epipactis helleborine* (Orchidaceae), one is a hololectin (EHA) and the other is a merolectin (EHMBP). EHA is extracted from the leaves of *Epipactis helleborine* and purified by affinity chromatography using immobilized mannose. It is not glycosylated and has a molecular weight of 25kDa and consisting of two identical subunits of 12.5kDa. The lectin occurs as a mixture of isolectins. EHMBA is purified by affinity chromatography and exists as a monomer of 14kda. The lectin agglutinates only rabbit erythrocytes but not human RBC. The EHA shows high inhibitory activity on HIV-1 and HIV-2 at concentrations of 0.04 μg ml l^{-1} (Balzarini *et al.*, 1992). The merolectin shows no hemagglutinating activity (Balzarini *et al.*, 1992). No further information is available on its applications and commercial availability.

Lectin from Listera Ovata (Loa)

The *Listera ovata*, commonly known as twayblade orchid, contains two different lectins *i.e.* one is a hololectin (LOA), whereas the other is a merolectin (LOMBP). Both the lectins are isolated by affinity chromatography on immobilized mannose. LOA is highly specific for mannose with a high specificity for α (1,3)- mannosidic linkages (Saito *et al.*, 1993). The terminal and the internal α (1,3)- mannosidic linkages of carbohydrate chain are recognized. LOA also reacts with various α - mannans and galactomannans from yeast, fungi and bacteria but not with α -glucans. It is not glycosylated with a molecular weight of 25kDa and consisting of two identical subunits of 12.5kDa. The lectin occurs as a mixture of isolectins (Saito *et al.*, 1993). LOA readily agglutinates rabbit RBC. The *Listera* lectin exhibits a modest mitogenic activity towards human lymphocytes (Van Damme *et al.*, 1994). Further, the lectin shows high inhibitory effects on HIV-1 and HIV-2 at concentrations of 0.3 and 0.1 μg ml l^{-1}, respectively. However, the merolectin does not exhibit the same effect (Balzarini *et al.*, 1992). Although it is not commercially available, but still the mannan and glycogen from yeast cells can be completely resolved by affinity chromatography on a column of immobilized LOA. Moreover, the retention of several high-mannose glycoproteins by LOA immobilized column suggests that this lectin can be a useful tool for purification and structural investigation of α-mannosyl-containing polysaccharides and glycoconjugates (Saito *et al.*, 1993).

Conclusion

A comparative analysis of the amino acid sequences of plant lectins revealed that there are four major sub-groups of structurally and evolutionary related proteins, which comprise the vast majority of all currently known lectins. These findings not only reduce the apparent homogeneity of the whole group of plant lectins but also suggest that plants have succeeded in evolving a vast array of carbohydrate- binding proteins from a small number of ancestral (lectin) genes.

This review clearly establishes the fact that the lectins are not only widely occurring plant proteins, but also

possess many – faceted activities, some of which have tremendous biomedical importance. Lectins occur in the roots, stems, leaves, seeds, and other vegetative structures of plant species of any age. Therefore, more research should be focused to isolate, purify and characterize these molecules, which promise to hold a vast therapeutic potential.

Incidentally, orchids, the second largest group of angiosperms, almost remained unexploited in this respect. Many of the orchid species are used as ethnomedicines from time immemorial for treatment of several human ailments. Therefore, more attention should be paid to this unique group of plants, which may add new dimentions to the field of lectinology.

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