

Plant Lectins

Lectins, a class of carbohydrate-specific proteins of non-immune origin that are commonly detected by their ability to agglutinate erythrocytes, are ubiquitous in nature^(1,2). The richest source of these proteins are plants, where they often are found in high levels in the seeds^(3,4). For example, the well known lectins concanavalin A, soybean agglutinin, pea lectin, favin and phytohemagglutinin (PHA) comprise 1-8% of the seed protein. Even higher levels (up to 40% of total protein) have been found in other plant tissues and organs, e.g. bark. Several hundreds of plant lectins have been purified by affinity chromatography on immobilized carbohydrates in 10-100 mg quantities and many have been thoroughly characterized; over 70 of these are from a single family, the *Leguminosae*. A number of these have been cloned and expressed in bacteria (see e.g. 5). Furthermore, the three dimensional structures of some ten plant lectins are now known, most of them also in complex with sugars⁽⁶⁾.

Lectins are classified into a small number of specificity groups – mannose, galactose, N-acetylglucosamine, N-acetylgalactosamine, L-fucose and N-acetylneuraminic acid – according to the monosaccharide which is the most effective inhibitor of the agglutination of erythrocytes or precipitation of polysaccharides or glycoproteins by the lectin. The lectins within each group may differ markedly in their affinity for the specific monosaccharide or its derivatives. Moreover, certain lectins combine more strongly with di-, tri-, and tetra-saccharides than with monosaccharides. In such oligosaccharides, the specific monosaccharide is usually present at the nonreducing end, although there are lectins that react with internally placed sugars as well. The association constants for the binding of monosaccharides and oligosaccharides to lectins are typically in the range 10^3 - 5×10^4 M⁻¹ and 10^5 - 10^7 M⁻¹ respectively. These values are of the same order of magnitude as those for the binding of haptens to antibodies and of substrates to enzymes.

Lectins exhibit an astonishing range of biological activities, all of which depend on their ability to bind carbohydrates specifically and reversibly. These activities have been best documented for plant lectins. As mentioned, they precipitate polysaccharides and glycoproteins from solution; they also combine with glycolipids. Some lectins react selectively with erythrocytes of different blood types or with certain lymphocyte subpopulations. Other are potent mitogens (polyclonal activators) of T or B lymphocytes, stimulating these cells to divide and to produce cytokines; furthermore they may induce lysis by cytotoxic T cells of a wide range of target cells, including tumor cells. A few plant lectins are highly toxic to mammalian cells.

FUNCTIONS

Despite the vast literature on plant lectins, little is known with certainty about their physiological functions. According to one hypothesis, they are involved in establishing the symbiosis between nitrogen-fixing microorganisms, primarily rhizobia, and leguminous plants⁽⁷⁾, a process of great importance in both the nitrogen cycle of terrestrial life and in agriculture. Another hypothesis assumes that the most likely function of lectins is in plant defense⁽⁸⁾.

The association between legumes and nitrogen-fixing bacteria is highly specific. For example, rhizobia that infect and nodulate soybeans cannot nodulate garden peas or white clover, and vice versa. The idea that lectins are responsible for this association is based primarily on the finding that in most cases a lectin from a particular legume – for example, soybean agglutinin – binds in a sugar-specific manner to the corresponding rhizobial species and not to bacteria that are symbionts of other legumes. Support for this theory came from studies of trifolin, a lectin isolated from clover seeds and root seedlings, which binds selectively to clover symbionts. It could be released from clover roots by sugars for which it is specific, suggesting that it associates with the root surface via its carbohydrate-binding site. Trifolin may thus act as a bridge between similar carbohydrates on both the root hair tips and the symbiotic rhizobia. Additional evidence for this theory was obtained when it was found that transgenic clover plants transfected with the pea lectin gene were nodulated by the *Rhizobium* symbiont of pea, which normally does not nodulate clover.



3-D structure of the *Erythrina coraliodendron* lectin in a complex with lactose. (Courtesy of The Glycobiology Institute, Oxford, UK.)

Still, the theory is not widely accepted, because of other contradictory findings. For instance, the pea symbiont binds to the root hairs not only of pea but also of other leguminous plants, such as *Canavalia ensiformis* and *Medicago sativa*. The latter, however, are not infected by this bacterium. Also, sugars specific for pea lectin did not inhibit the attachment of this symbiont to the root hairs.

The toxicity of various plant lectins for animals and their growth inhibitory effects on fungi, are the basis of the assumption that they function as defense agents. Feeding experiments in animals have shown that purified PHA is toxic and that it probably acts by binding to the intestine and interfering with its normal activities. It was reported that the lectin is also toxic to plant pathogenic insects, but this effect was subsequently traced to the presence of other toxic protein contaminants in the PHA preparations used.

Research carried out in the 1970's demonstrated that wheat germ agglutinin, (WGA) that binds chitin and its oligosaccharides, as well as some other plant lectins with different specificities, possess antifungal activity. Reinvestigation of this activity of WGA revealed that it is very likely due to contamination of the lectin by trace amounts of chitinase, a potent fungitoxic protein. However, it was later shown that the stinging nettle lectin, which was devoid of chitinase, had strong antifungal properties. Thus, in spite of some doubts, the idea that plant lectins are defense molecules continues to attract attention.

APPLICATIONS

The ability to recognize particular carbohydrates and the biological activities of plant lectins mentioned, combined with the ease of their preparation, make them excellent tools for chemical and biological research of carbohydrate-containing compounds and cells as well as for clinical uses.

The main application of native lectins is for precipitation and agglutination reactions or for mitogenic stimulation. For most purposes, however, lectin derivatives are required. Thus, lectins derivatized with fluorescent dyes, gold particles, biotin or enzymes are employed for detection of glycoconjugates on electrophoretograms and gel blots⁽⁹⁾, in tissue sections, on cells and subcellular organelles⁽¹⁰⁾, and in studies of intracellular pathways of protein glycosylation; when native lectins are used for the same purposes, they can be detected by immunological probes. Since modifications in content, distribution, and accessibility of cellular and extracellular glycoconjugates are often associated with pathological processes, much effort is invested in screening lectins for their potential as diagnostic reagents in clinical situations. Lectin binding is routinely employed to demonstrate that membrane receptors for hormones, growth factors, neurotransmitters and toxins are glycoconjugates.

Immobilized lectins, e.g. covalently bound to Sepharose™, are indispensable for the isolation and purification by affinity chromatography of glycoproteins, glycopeptides, and oligosaccharides^(11,12). The high specificity of lectins permits the resolution of even closely related compounds, such as glycoforms of a glycoprotein or glycopeptides that differ only slightly in the structure of their carbohydrate chains. These proteins are also used to obtain structural information on polysaccharides and glycoconjugates. The toxicity of lectins is the basis for their use for the selection of lectin-resistant mutants of animal cells⁽¹³⁾. Such cells have greatly contributed to our knowledge of the biosynthesis of glycoproteins and the roles of their carbohydrate moieties.

Mouse or human cortical (immature) thymocytes can be separated from the medullary (immature) ones by agglutination of the former cells by peanut agglutinin. Although the presence of the two cell populations in the thymus has been recognized for some time, selective agglutination by the lectin has provided facile access to the individual thymocyte subpopulations and made it possible to examine in vitro their developmental and functional relationships⁽¹⁴⁾.

Soybean agglutinin may be employed for the fractionation of mouse splenocytes into B and T cells by agglutination of the former subpopulation. However, the most important application of this lectin is for the removal of immunocompetent lymphocytes from human bone marrow used for transplantation. Bone marrow from haploidentical donors, purged by soybean agglutinin, is routinely used for transplantation of children born with severe combined immune deficiency ("bubble children") with a high rate of success (about 70%). Lectin purging is also employed on an experimental basis in bone marrow transplantation of leukemic patients, alongside other techniques for T cell depletion, (e.g. monoclonal antibodies). It was used in Moscow in May 1986, in attempts to save the lives of several of the lethally irradiated victims of the Chernobyl nuclear accident. The two surviving victims of the accident received bone marrow that had been treated with soybean agglutinin.

A longstanding application of plant lectins is in blood typing⁽¹⁵⁾. For instance, the lectins from *Lotus tetragonolobus* and *Ulex europaeus* serve to identify blood type O cells, as well as secretors, individuals who secrete blood group substances in their saliva and other body fluids. The lectin from *Dolichos biflorus* is used to distinguish between A₁ and A₂ subgroups and that from *Vicia graminea*, specific for blood type N, to differentiate between M and N cells. In addition, peanut agglutinin, specific for the T antigen, is employed in the differential diagnosis of "polyagglutination", a condition accompanying certain bacterial and viral infections, in which human erythrocytes become agglutinable by antibodies normally present in the sera of nearly all adults.

Plant Lectins

Mitogenic stimulation by lectins^(1,2) provides an easy and simple means to assess the immunocompetence of patients suffering from a diversity of diseases, for example AIDS, and to monitor the effects of various immunosuppressive and immunotherapeutic manipulations. It has been used to examine the effect of stress, both physical and psychological, on the immune system, e.g. sport, weightlessness in space, bereavement. It is also employed for the preparation of chromosome maps for karyotyping, sex determination, and detection of chromosomal defects, since chromosomes are easily visualized in the stimulated cells.

REFERENCES

1. Sharon, N. and Lis, H. (1989) *Lectins*, Chapman and Hall, London
2. Liener, I.E., Sharon, N. and Goldstein, I.J., eds., (1986) *The Lectins – Properties, Functions and Applications in Biology and Medicine*, Acad Press, Boca Raton, FLA
3. Sharon, N. and Lis, H. (1990) *FASEB J.* **4**, 3198-3208
4. Pusztai, A. (1991) *Plant Lectins*, Cambridge Univ Press, Cambridge
5. Arango, R., Adar, R., Rozenblatt, S. and Sharon, N. (1992) *Eur. J. Biochem.* **205**, 575-581
6. Sharon, N. (1993) *TIBS* **18**, 221-226
7. Kijne, J., Diaz, C., dePater, S. and Lugtenberg, B. (1992) *Adv. Lectin Res.* **5**, 15-50
8. Chrispeels, M.J. and Raikhel, N.V. (1991) *Plant Cell* **3**, 1-9
9. Gershoni, J. (1988) *Methods Biochem. Anal.* **33**, 1-58
10. Spicer, S. S. and Schulte, B. A. (1992) *J. Histochem Cytochem* **40**, 1-38
11. Osawa, T. and Tsuji, T. (1987) *Ann. Rev Biochem* **56**, 21-42
12. Debray, H. and Montreuil, J. (1991) *Adv. Lectin Res.* **4**, 51-96
13. Stanley, P. (1987) *Trends Genetics* **3**, 77-81
14. Sharon, N. (1983), *Adv. Immunol.* **34**, 213-298
15. Kilpatrick, D. C. and Green, C. (1992) *Adv. Lectin. Res* **5**, 51-94