The structure Biology and Application of Phytohemagglutinin (PHA) in Phytomedicine: With special up-to-date references to lectins

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ABSTRACT

Lectins first discovered more than 100 years ago in plants, they are now known to be present throughout nature. Phytohemagglutinin (PHA), the lectin extract from the red kidney bean (*Phaseolus Vulgaris*), contain potent, cell agglutinating and mitogenic activities. They play a role in biological recognition phenomena involving cells and proteins towards medical applications. The present article is a brief review of the history of lectin in nature. By reviewing the web-based search for all types of peer review articles published, was initiated using ISI web of Sciences and Medline / PubMed, and other pertinent references on websites about lectins. Here, we present a brief account of 100-plus years of lectin research and show how these proteins have become the focus of intense interest for biologists and in particular for the research and applications in medicine. Phytohemagglutinin, has been widely used for mitotic stimulation to human lymphocytes, cell arrest, or apoptosis, potential sources for developing novel pharmaceutical preparation   and intensive interest for health care services, biologist and phytomedicine research can be considered.

Keywords: Lectins; PHA; Significant role; Cell; Biology; Medicine.

INTRODUCTION

Toward the end of the 19th century, evidence started to accumulate for the presence in nature of proteins possessing the ability to agglutinate erythrocytes. Such proteins were referred to as hemagglutinins, or phytoagglutinins, because they were originally found in extracts of plants. It is generally believed that the earliest description of such a hemagglutinin was by Peter Hermann [1]. This hemagglutinin, which was also highly toxic, was isolated by Stillmark from seeds of the castor tree (*Ricinus communis*) and was named ricin. Subsequently, H Hellin, at Tartu University, Stonia demonstrated the presence of a toxic hemagglutinin, abrin, in extracts of the jequirity bean (*Abrus precatorius*). Ricin and abrin soon became commercially available, to employ them as model antigens for immunological studies. Mice were rendered immune to a lethal dose of ricin or abrin by repeated small, subcutaneous injections of the lectin and that anti-ricin did not protect the animals against the toxic effects of abrin, nor did anti-abrin protect against ricin. This provided clear evidence for the specificity of the immune response. Immunity to the toxins is transferred from a mother to her offspring by blood during pregnancy and by milk after birth. By studying the inhibitory effect of the anti-ricin immune serum on the agglutinating activity of ricin, there is a quantitative relationship between the amount of antiserum and that of antigen, it could neutralize and on this basis performed the first quantitative determination of an antibody in vitro. These studies thus demonstrated the specificity of the antibody response, the

phenomenon of immunological memory, and the transfer of humoral immunity from a mother to her offspring. More recently research concluded that, reversed micelles were used to extract lectin from red kidney beans and factors affecting reverse micellar systems (pH value, ionic strength and extraction time) were studied. The optimal conditions were extraction at pH 4–6, back extraction at pH 9–11, ion strength at 0.15 M NaCl, extraction for 4–6 minutes and back extraction for 8 minutes [1] .

Most lectins are non-enzymatic in action and non-immune in origin. Lectins occur ubiquitously in nature. They may bind to a soluble carbohydrate or to a carbohydrate moiety that is a part of a glycoprotein or glycolipid. They typically agglutinate certain animal cells and/or precipitate glycoconjugates [2].

Sugar binding and blood type specificity

In 1919, James B. isolated from jack bean (Canavalia ensiformis) a crystalline protein that he named concanavalin A and in this way obtained a pure hemagglutinin for the first time. However, nearly two decades passed before [3] reported that concanavalin A agglutinates cells such as erythrocytes and yeasts and also precipitates glycogen from solution. They further showed that hemagglutination by concanavalin A was inhibited by sucrose, demonstrating for the first time the sugar specificity of lectins. With much foresight, they suggested that the hemagglutination induced by concanavalin A might be a consequence of a reaction of the plant protein with carbohydrates on the surface of the red cells.

Already the early results indicated some selectivity in the ricin-induced agglutination of red cells from different animals. This observation was corroborated and further extended by Karl Landsteiner the discoverer of the human A, B, and O blood groups in 1900. Nearly a decade later he reported that the relative hemagglutinating activities of various seed extracts were quite different when tested with red blood cells from different animals [4]. Because of this specificity, Landsteiner concluded that the actions of plant hemagglutinins resemble antibody reactions in all essentials.

The 1940s C. Boyd and Karl O. found that crude extracts of the lima bean*, Phaseolus limensis*,

and the tufted vetch, *Vicia cracca*, agglutinated blood type A erythrocytes but not blood type B or O cells, whereas an extract of the asparagus pea, Lotus tetragonolobus, agglutinated specifically blood type O erythrocytes. Olavi Mäkelä (1957), examined in 1954–1956 extracts from seeds representing 743 plant species and 165 genera, all of the family *Leguminosae*, and detected hemagglutinating activity in more than one-third of them; close to one-tenth of the hemagglutinins exhibited blood type specificity. Although several of the latter were specific either for blood type O or type A, or both type A and B erythrocytes, and one, from *Dolichos biflorus*, reacted much better with A_1 erythrocytes than with A_2, only the extract from *Griffonia simplicifolia* (previously known as *Bandeiraea simplicifolia*) exhibited almost exclusively type B specificity. Since then, additional hemagglutinins specific for blood types A and O (but not B) have been discovered, as well as several for other blood types, such as N (*Vicia graminea* lectin), T (Peanut agglutinin, PNA) and Tn (the lectins of *Vicia villosa* and *Moluccella laevis*).

The blood type–specific hemagglutinins played a crucial role in early investigations on the structural basis of the specificity of the antigens associated with the ABO blood group system. In the 1950s, Walter Morgan and Winifred. Watkins found that the agglutination of type A red cells by lima bean lectin was best inhibited by α-linked N-acetyl-D-galactosamine and that of type O cells by the lectin of L, tetragonolobus was best inhibited by α-linked L-fucose. They concluded that α-N-acetyl-D-galactosamine and α-L-fucose are the sugar determinants conferring A and H(O) blood group specificity, respectively. Both conclusions have been substantiated by subsequent investigations [6]. The pioneering work of Watkins and Morgan was among the earliest evidence for the presence of sugars on cell surfaces and their potential roles as identity markers, an accepted theme in modern glycobiology. It took a while, however, before the counter receptors for surface sugars, that is, the endogenous lectins that recognize these sugars, were identified, the first being the mammalian hepatic asialoglycoprotein receptor .

The ability of plant agglutinins to distinguish between erythrocytes of different blood types led [7] to propose for them the name lectins, from

the Latin legere. This term was generalized by all to embrace every sugar-specific agglutinins of nonimmune origin, irrespective of source and blood type specificity [8].

### *Mitogenic stimulation of lymphocytes and agglutination of cancer cells*

Two major discoveries made in the early 1960s were instrumental in bringing lectins into the limelight. The first of these was by Peter C. Nowell (1960) [9] who found that the lectin of the red kidney bean (*Phaseolus vulgaris*), known as phytohemagglutinin (PHA), is mitogenic, that is, it possesses the ability to stimulate lymphocytes to undergo mitosis [10]. This discovery had a revolutionary impact on immunology in that it shattered the view, held until then, that lymphocytes are dead-end cells incapable of dividing or differentiating further. Within a short time, several other lectins were proven to be mitogenic. Of special significance was the finding that concanavalin A acts as a mitogen because, in contrast to PHA, its activity could be inhibited by low concentrations of monosaccharides, for example, mannose. This finding provided proof that mitogenic stimulation is the result of binding of lectins to sugars on the surface of the lymphocytes and was among the earliest demonstrations for a biological role of cell surface sugars. Mitogenic lectins soon became tools for the study of signal transmission into cells and for the analysis of the biochemical events that occur during lymphocyte stimulation in vitro. A most valuable outcome of such studies was the discovery in the 1970s by Robert C. Gallo of T cell growth factor, now known as interleukin-2, in conditioned medium of normal human lymphocytes stimulated by PHA [11]. The subunits of PHA are of two different types, designated leucocyte reactive (L) and erythrocyte reactive (E). L has high affinity for lymphocyte surface receptors but little for those of erythrocytes and is responsible for the mitogenic properties of the isolectins. The E is responsible for the erythrocyte agglutinating properties. Phytohemagglutinin -P is the protein form of   PHA-M is the mucoprotein form of these isolectin [12]. It was known to exist some individual differences in response to PHA and the limitation of only T-lymphocyte stimulation by PHA. Therefore, Pokeweed mitogen (PWM) which was known to stimulate T and B-lymphocytes, and some other mitogens such as Concavalin A(Con-A), lipopolysaccharide (LPS), Wheat Germ Agglutinin (WGA) and Soybean Agglutinin (SBA) were used and compared their mitotic stimulating effects in single use and also combined use of these mitogens (figures 1-6) [13]. One of the mitogens, phytohemagglutinin (PHA), has been widely used for mitotic stimulation to human lymphocytes, and several different types of PHA, such as PHA-P, M, W and others were compared its ability to induce mitoses were presented by other workers [14-17].

The second discovery was made by J. C. Aub [18]. They found that wheat germ agglutinin has the ability to preferentially agglutinate malignant cells. Such investigations provided early evidence that changes in cell surface sugars are associated with the development of cancer and led to the assumption that high susceptibility to agglutination by lectins was a property shared by all malignant cells.

Until the early 1970s, the presence of hemagglutinins had been reported in numerous organisms, primarily plants, but only very few had been purified, almost all by conventional techniques. In addition to Concavalin A, they included the plant lectins from soya beans, green peas, Dolichos biflorus seeds, wheat germ, and mushroom (Agaricus campestris) [19] and the animal lectins of eel [20], snail [21], and horseshoe crab [22]. The pace of lectin isolation increased dramatically with the introduction of affinity chromatography for lectin purification [23].

In an addition to the growing knowledge of lectins is mammalian lectin is dectin-1, a β-glucan receptor, is identified by Gordon and Brown [24]. A new type of plant root lectin found in different leguminous plants but not in plants of different family [25].
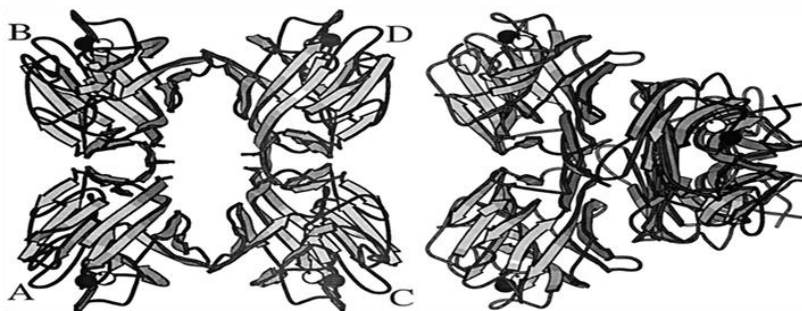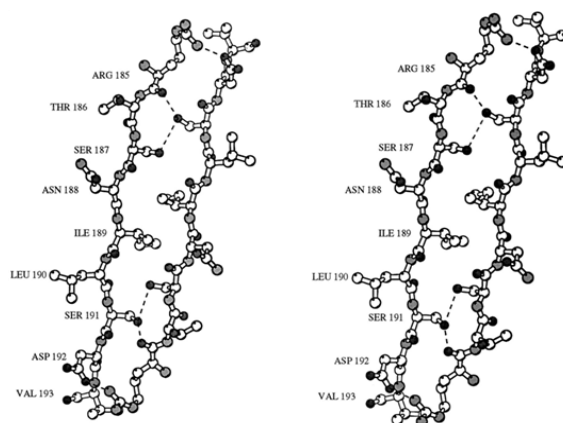
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Figure 1.Comparison between ConA and PHA-L. The PHA-L tetramer is shown on the left, the ConA tetramer is shown on the right. The left dimers in both tetramers have the same orientation to emphasize the difference in dimer-dimer packing between PHA-L and ConA. The central channel running between the two dimers in PHA-L is clearly visible.
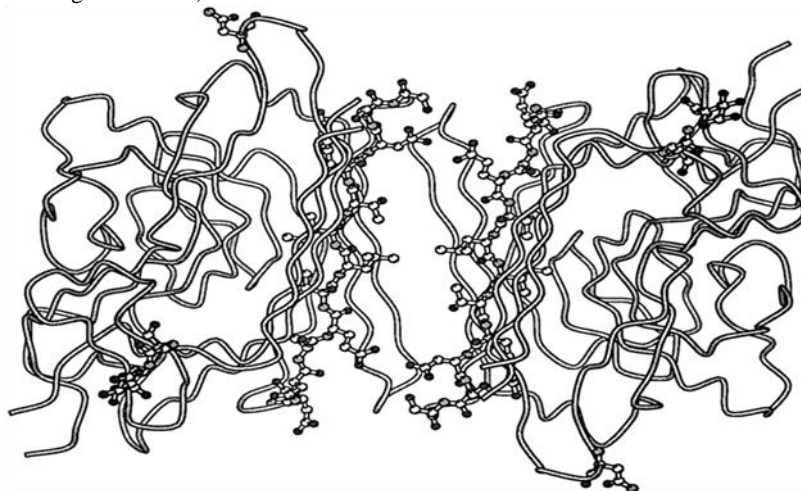A stereo figure of the dimer-dimer interface in the SBA tetramer.
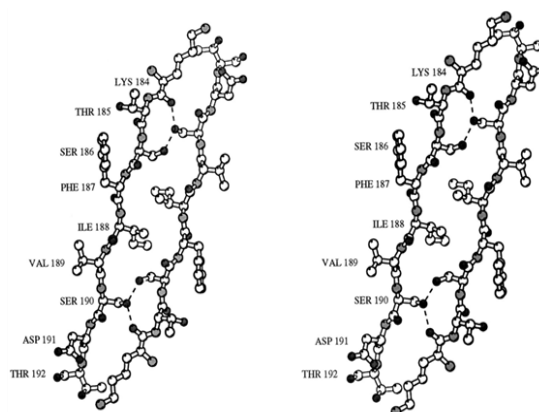
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Figure 2.A stereo figure of the dimer-dimer interface in the SBA tetramer. The interface is similar to the dimer-dimer interface in PHA-L. The 2-fold axis is approximately positioned in the center of the figure. As in PHA-L, the side chains of 2 Ser (Ser-191 and −187) and 1 Ile residue (Ile-189) intercalate. The main difference with PHA-L is the substitution of Lys-184 in PHA-L by Arg-185 in SBA. The Arg-185 is involved in an additional hydrogen bond with Asp-192. The six hydrogen bonds (Arg-185A O-Ser-191C OG, Arg-185A NH1-Asp-192C OD1, Ser-187A OG-Ser-191C OG, Ser-191A OG-Arg-185C O, Ser-191A OG-Ser-187C OG, Asp-192A OD1-Arg-185C NH1) across the interface are visualized as dashed lines.
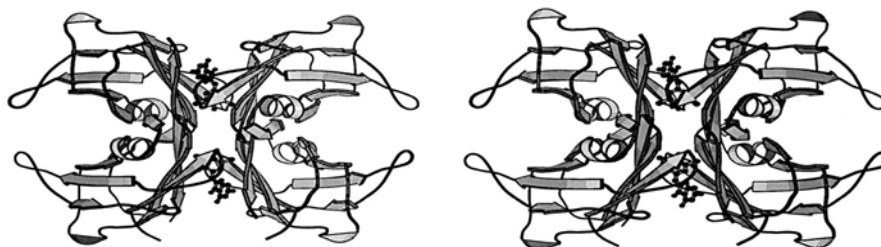

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Figure 3. A stereo figure of the dimer-dimer interface in the SBA tetramer. The interface is similar to the dimer-dimer interface in PHA-L. The 2-fold axis is approximately positioned in the center of the figure. As in PHA-L, the side chains of 2 Ser (Ser-191 and −187) and 1 Ile residue (Ile-189) intercalate. The main difference with PHA-L is the substitution of Lys-184 in PHA-L by Arg-185 in SBA. The Arg-185 is involved in an additional hydrogen bond with Asp-192. The six hydrogen bonds (Arg-185A O-Ser-191C OG, Arg-185A NH1-Asp-192C OD1, Ser-187A OG-Ser-191C OG, Ser-191A OG-Arg-185C O, Ser-191A OG-Ser-187C OG, Asp-192A OD1-Arg-185C NH1) across the interface are visualized as dashed lines.
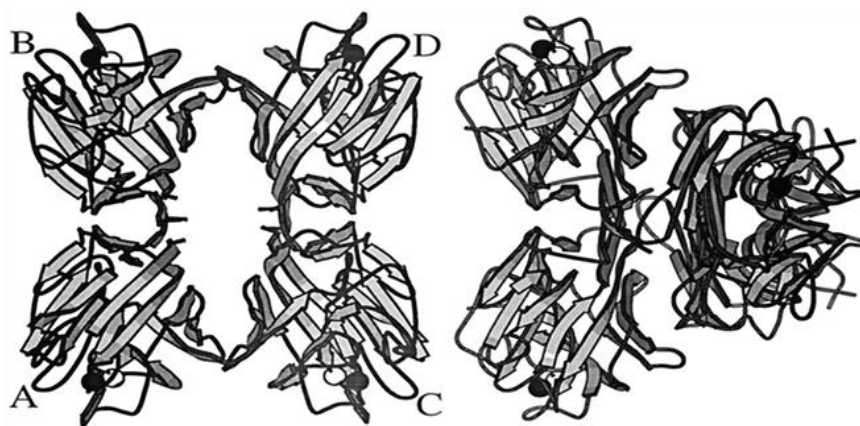

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Figure 4. A view from the center of the molecule to the dimer-dimer interface on the putative adenine binding site between dimers A and C. The strand that contains the photoaffinity labeled residues in both monomers is shown as a ball-and-stick representation. The two glycosylated residues per monomer (Asn-12 and Asn-60) are also shown in ball-and-stick representation, together with the GlcNAc residue bound to Asn-12.

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Figure 5. Stereo figure of the transthyretin tetramer complexed with 3′,5′-dibromo-2′,4,4′,6-tetrahydroxyaurone, a flavone derivate. The ligand is bound in the central hole that runs through the molecule, through interactions with side chains of the residues that make up the flanking β-strands (mainly Ser, Thr, Ala, and Leu). The binding site possesses 2-fold symmetry. The central hole is approximately 10 Å wide.

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Figure 6. Comparison between ConA and PHA-L. The PHA-L tetramer is shown on the left, the ConA tetramer is shown on the right. The left dimers in both tetramers have the same orientation to emphasize the difference in dimer-dimer packing between PHA-L and ConA. The central channel running between the two dimers in PHA-L is clearly visible.

From primary to 3D structures

Some studies ranged from the determination of the main physicochemical parameters of lectins to complete amino acid sequencing and elucidation of their 3D structure. Until the advent of recombinant techniques, determination of the primary structure of lectins proceeded rather slowly, and by the end of that decade the complete sequences of only half a dozen lectins, all from plants, were known. In this case, too, concanavalin A led the field, being the first lectin whose primary sequence has been established [26]. Concurrently, Edelman's group and independently Karl Hardman solved the 3D structure of concanavalin A by high resolution X-ray crystallography [27]. This was soon followed by the determination of the structure of WGA as well as of its complexes with its ligands (N-acetylneuraminic acid and β4-linked N-acetylglucosamine oligomers) by Christine Schubert Wright even before the complete amino sequence of this lectin had become available [28].

The availability of the primary structure of numerous lectins allowed the identification of homologies between the sequences of lectins from taxonomically related sources [29]. By the end of the following decade, homologies were found also for lectins from other families, such as the galectins and the C-type (Ca^{2+} requiring) lectins [30].

During the past 20 years, the number of lectin primary and 3D structures has increase dramatically, with some 200 of the latter having been elucidated (www.cermav.cnrs.fr/lectines).

In addition, many structures of lectin–carbohydrate complexes have been solved. Quite surprisingly, remarkable similarities have been noticed between the tertiary structures of lectins from diverse sources, in spite of the lack of primary sequence similarities (Figure 7). One such common tertiary structure, first observed in the legume lectins, and referred to as the lectin fold, consists characteristically of an elaborate jelly roll, derived from antiparallel β-strands, arranged as two β-sheets [31]. This fold has been found in the legume lectins, the galectins, and in several other animal lectins, such as the pentraxins [32] and ERGIC-53 [33,34], as well as in nonlectin molecules, for example, several *glycosidases*, among them *Vibrio cholerae sialidase*.

Starting in the late 1980s, considerable information has become available, by X-ray crystallography and site-directed mutagenesis, of the chemical groups on the lectin and on the carbohydrates that interact with each other and of the types of bond formed, primarily hydrogen bonds and hydrophobic interactions. It has been concluded that lectins recognize sugars in diverse ways, just like other proteins recognize their ligands [35].

Carbohydrate recognition domains

Based on an analysis of the then known amino acid sequences of animal lectins, Kurt Drickamer from Columbia University (New York) proposed in 1988 that the carbohydrate-binding activity of most of them resides in a limited polypeptide segment, designated by him as the carbohydrate-

recognition domain (CRD) [30]. He named the CRD found in the galectins S-CRD and that found in C-type lectins C-type CRD. By now several types of CRD have been discerned, in addition to those just mentioned, each of which shares a pattern of invariant and highly conserved amino acid residues at a characteristic spacing. On this basis it was possible to divide the majority of the animal lectins into structurally related families and superfamilies, the most widely occurring of which is that of the C-type lectins (CTLs). Other families of special interest are the P-type *lectins* and the *siglecs*. The majority of the CTLs are large, asymmetric transmembrane glycoproteins, in which the CRD is attached to a variable number of structurally and functionally different polypeptide domains. In contrast, the galectins are generally small, soluble, nonglycosylated proteins and, unlike the CTLs, do not require Ca^{2+} for their activity. Members of the CTL superfamily are grouped into three families—*selectins* (the most celebrated one), *collectins*, and *endocytic lectins*. The story of the *selectins* started with attempts to elucidate the molecular basis of lymphocyte homing. These attempts greatly benefited from the availability of an in vitro assay for measuring the interaction of lymphocytes with postcapillary high-endothelial venules (HEVs), a known site of lymphocyte exit from the blood stream [36]. Using this assay, which reflects the in vivo homing of lymphocytes, Eugene C. Butcher and colleagues at Stanford University obtained a monoclonal antibody (MEL-14) against a murine lymphocyte antigen [37]. The antibody inhibited the binding of the lymphocytes to HEV in vitro and their homing in vivo, suggesting that the MEL-14 antigen has a direct role in these phenomena. From inhibition experiments of the lymphocyte-HEV binding, Steven D. Rosen and Loyd M Stoolman have concluded that sugars of the endothelial cell might also be involved in this binding and that the lymphocytes may have a membrane-bound lectin with specificity for fucose and Man-6-P [38]. This lectin was subsequently shown to be identical with the MEL-14 antigen.

In 1987 Bevilacqua and co-workers [39] have developed two monoclonal antibodies that identified a second cell-surface antigen, designated ELAM (endothelial-leukocyte adhesion molecule)-1, expressed on stimulated human endothelial cells but not on unstimulated ones [39]. Another vascular cell adhesion molecule was originally isolated from activated platelets independently by Rodger McEver [40] and by Bruce and Barbara C. Furie [41,42], and designated GPM-140 and PADGEM, respectively. These three cell adhesion molecules, collectively known for a while as LEC-CAMS, were identified as a discrete family of CTLs after the virtually simultaneous publication in 1989 of their primary sequences [43], these go now under the names *L-selectin, E-selectin, and P-selectin*, respectively (reviewed in Lasky, 1995) [44]. They were all shown to have a similar domain structure, with an extracellular part that consists of an amino terminal CRD, an epidermal growth factor–like domain, and several short repeating units related to complement-binding protein. They bind specifically to with both fucose and sialic acid [45]. The selectins recognize the carbohydrate ligands only when the latter are present on particular glycoproteins, such as cell surface mucins, pointing to the role of the carrier molecule in *lectin-carbohydrate* interactions; one of the best characterized of such carriers is the *P-selectin* glycoprotein ligand [46].

The paradigm of the endocytic lectins is the mammalian hepatic asialoglycoprotein receptor is the matter of interest. The *collectins,* represented by the soluble mannose-binding proteins of mammalian serum and liver, first detected by chance as a contaminant of a preparation of *α-mannosidase* from human liver [47], subsequently purified and characterized by Toshiaki Kawasaki and Ikuo Yamashina at Kyoto University, Japan [48] are characterized by an NH_2-terminal collagen-like stretch of repeating *Gly-X-Y-* triplets (where X and Y are any amino acid). The structural unit of the mannose-binding proteins is a trimer of identical subunits with a triple-stranded collagen helix and three CRDs [49]. A different kind of CRD has been identified in the siglecs. This family of sialic acid–binding Ig-like lectins, a member of the Ig superfamily, was discovered when the cloning of a macrophage lectin-like adhesion molecule named sialoadhesin (*siglec-1*) revealed striking structural similarities to a B cell restricted member of the Ig super family, CD22 (*siglec-2*) and to two other members of the Ig super family, CD33 (*siglec-3*) and the myelin-

associated glycoprotein (*siglec-4*) [50]. Members of this family, 11 of which have been identified in humans, are type 1 transmembrane proteins with an extracellular part consisting of a CRD-containing N-terminal V-set Ig-like domain, followed by variable numbers of C2-set Ig-like domains. Except for myelin-associated glycoprotein (*siglec-4*), exclusively expressed in the nervous system, they are all found on cells of the hematopoietic system. Each *siglec* has a distinct expression pattern in different cell types, indicating that they perform highly specific functions.

A last decade addition to the growing list of mammalian lectins is dectin-1, a *β-glucan* receptor. It is a small type II transmembrane receptor containing one CRD, which recognizes $\beta 1,3$ and/or $\beta 1,6$-glucans and intact yeasts.

In protection and symbiosis

The question of the possible physiological role of lectins has intrigued investigators from the start and focused on plant lectins, which for long time were virtually the only ones known (reviewed by Etzler, 1986) [51]. It was speculated, for example, that lectins may function as antibodies to protect plants against harmful soil bacteria, control seed germination, or be involved in the transport and storage of sugars.

In an extensive study, 11 purified lectins representing the major carbohydrate specificity groups were all found to cause growth disruption during germination of spores of *Neurospora crassa, Aspergillus amstelodami,* and *Botryodiplodia theobromae* [52]. It was also shown that recombinant *Urtica dioica* agglutinin that has a similar specificity to that of WGA [53] inhibited the growth of fungal phytopathogens. The idea that lectins may be involved in the protection of plants against pathogenic microorganisms was basically on the research made at Rehovot that WGA, PNA, and SBA inhibited the sporulation and growth of fungi such as *Trichoderma viride, Penicilium notatum,* and *Aspergillus niger* [54]. Potato lectin was subsequently shown to act in a similar manner on *Botrytis cinerea*, another fungal Phytopathogen.

The proposal that lectins are responsible for the specific association between nitrogen-fixing *rhizobia* and leguminous plants, which provides the plant with the needed nitrogen, was advanced nearly three decades ago [55]. It was based on the finding that a lectin from a particular legume bound in a carbohydrate-specific manner to the surface polysaccharides or *lipopolysaccharides* of the corresponding rhizobial species but not to bacteria that are symbionts of other legumes. For instance, SBA agglutinated most strains of *Bradyrhizobium japonicum* that nodulate soybeans but not nonnodulating bradyrhizobial strains. The suggestion has therefore been made that *rhizobial* attachment to plant roots occurs by interaction between the bacterial surface carbohydrates and lectins present in the roots of the leguminous plants. Several lines of soybeans with no detectable lectins in their seeds or vegetative tissues were nodulated normally by the corresponding *rhizobial symbiont*.

Application of the techniques of molecular genetics gave results that bolstered the lectin recognition hypothesis but did not fully settle the controversy [56].

Recognition molecules

In a broader sense, the foregoing discussion implies that lectins possess the ability to act as recognition molecules inside cells, on cell surfaces, and in physiological fluids (Figure 2). This is in fact the current view of the biological function of lectins, which also evolved during the 1978 [57].
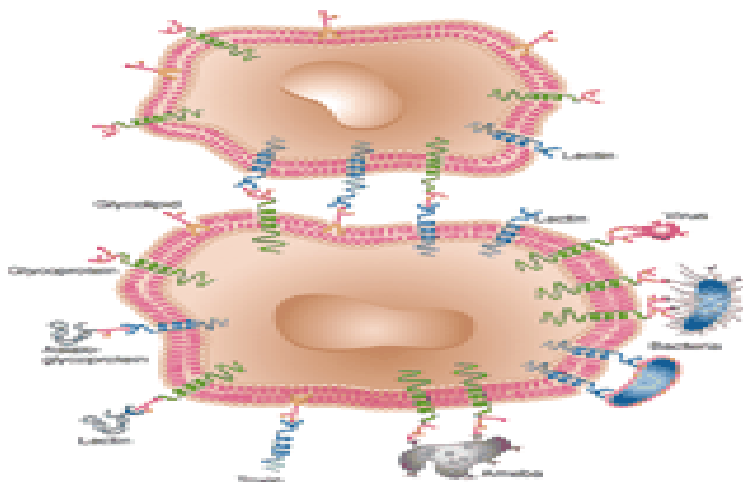
Figure 7. Cell surface lectin–carbohydrate interactions. Lectins serve as means of attachment of different kinds of cell as well as viruses to other cells. In some cases, cell-surface lectins bind particular glycoproteins (e.g., asialoglycoproteins), whereas in other cases the carbohydrates of cell surface glycoproteins or glycolipids serve as sites of attachment for biologically active molecules that themselves are lectins (e.g. carbohydrate-specific bacterial and plant toxins, or galectins).

Functions in animals

The occurrence of hemagglutinins in animals was noted quite early, almost all in invertebrates or lower vertebrates, but until the middle of the 1970s, only the three of these mentioned (of eel, snail, and horseshoe crab) were isolated and characterized. The first of the animal lectins shown to be specific for a sugar (L-fucose) was from the eel [58]. The isolation in 1974 of the first mammalian lectin, the galactose-specific hepatic asialoglycoprotein receptor, was an outcome of the investigation [59]. At the same time, Vivian Teichberg reported [60] the isolation from the electric eel of the first member of the family of the β-galactose-specific lectins, designated galectins [61], of which over a dozen members have by now characterized.

Lectins serve many different biological functions in animals, from the regulation of cell adhesion to glycoprotein synthesis and the control of protein levels in the blood. They may also bind soluble extracellular and intercellular glycoproteins. Some lectins are found on the surface of mammalian liver cells that specifically recognize galactose residues. It is believed that these cell-surface receptors are responsible for the removal of certain glycoproteins from the circulatory system. Another lectin is a receptor that recognizes hydrolytic enzymes containing mannose-6-phosphate, and targets these proteins for delivery to the lysosomes. I-cell disease is one type of defect in this particular system. Lectins are also known to play important roles in the immune system by recognizing carbohydrates that are found exclusively on pathogens, or that are inaccessible on host cells. Examples are the lectin complement activation pathway and mannose-binding lectin.

Use in medicine and basic sciences research

In recent years, a great number of lectins with in vivo and in vitro antiproliferative properties against cancer cells have been isolated and characterized. Phytohemagglutinin has the potential to induce closer contacts between adjuscent cell memberanes; it is an N-aceteylgalactosamine/ galactose sugar-specific lectin with wide variety of biological activities [62]. Phytohemagglutinin has been successfully used for memberne-induced fusion in human oocytes [63], bovine oocyt [64], and caprine oocytes [65]. It was the first direct evidence for the involvement of bacterial lectins in the initiation of infection, the basis for the present attempts in academia and industry to apply carbohydrates for antiadhesion therapy of such diseases reviewed by Mulvey and co-workers [66].

Urinary tract infection in mice by mannose-specific Escherichia coli could be prevented by methyl α-D-mannoside [67]. It was the first direct evidence for the involvement of bacterial lectins in the initiation of infection, the basis for the present attempts in academia and industry, to apply carbohydrates for antiadhesion therapy of such disease. Itzhak Ofek, demonstrated that the mannose-specific bacterial surface lectins may also mediate attachment of the bacteria to

phagocytic cells in the absence of opsonins, leading to engulfment and killing of the bacteria. This process, another example of innate immunity, which we named lectinophagocytosis, may be of importance in the clearance of bacteria from nonimmune patients or from opsonin-poor sites, such as renal medulla or the peritoneal cavity [68]. Additional lectins have been implicated in innate immunity. A prominent example is the mannose-specific receptor present on the surface of macrophages; it binds infectious organisms that expose mannose-containing glycans on their surface, leading to their ingestion and killing. Another, recently discovered one, is dectin-1, specific for β1,3 and/or β1,6-glucans, present on fungi. A similar function, albeit by a different mechanism, is performed by the soluble mannose-binding lectins (MBLs) of mammalian serum and liver, These proteins bind to *Oligomannosides* of infectious microorganisms, causing activation of complement without participation of antibody, and subsequent lysis of the pathogens, thus acting in innate immunity. The spatial arrangement of the CRDs in the MBLs provides a structural basis for their ability to bind ligands with repetitive, mannose-rich structures, such as found on fungal and microbial surfaces, but not to the oligomannose units of mammalian glycoproteins [49].

The discovery of the selections and the demonstration that they play a crucial role in the control of lymphocyte homing and of leukocyte trafficking to sites of inflammation was a landmark in lectin research. Indeed, the selections provide the best paradigm for the role of sugar–lectin interactions in biological recognition. 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. Prevention of adverse inflammatory reactions by inhibition of leukocyte–endothelium interactions, another application of anti adhesion therapy, has become a major aim of the biomedical, medical and pharmacological industry. There are also indications that the selection may function in the spread of cancer cells from the main tumor to other sites in the body and that by blocking their sugar-binding sites it may be possible to prevent the formation of metastases.

From the late 1980s, evidence started to accumulate that several lectins of different types direct intracellular glycoprotein traffic, by acting as chaperones and sorting receptors in the secretory pathway. *Calnexin,* a membrane-bound lectin of the endoplasmic reticulum (ER), functions in parallel with calreticulin, its soluble homolog, as part of a quality control system that ensures proper folding of glycoproteins destined to the cell surface. The mannose-specific intracellular lectin, ERGIC-53, first identified as a resident of the ER–Golgi intermediate compartment protein [69] carries a specific subset of nascent glycoproteins between the two compartments. Two distinct mannose-6-phospate receptors, the only members of the P-type lectin family, mediate the targeting of newly synthesized hydrolases from the rough ER to the lysosomes [70]. Both receptors bind their ligands, oligosaccharides bearing terminal Man-6-P residues, most efficiently at pH 6–7, allowing them to interact with hydrolases decorated with such oligosaccharides in the trans-Golgi network, and to release them in the more acidic environment of the lysosomes.

The *galectins* are believed to act as modulators of cell–substratum interactions and to be essential for the normal differentiation and growth of all multicellular animals. They are capable of inducing cell proliferation, cell arrest, or apoptosis (physiological cell death) and have been implicated in organ morphogenesis, tumor cell metastasis, leukocyte trafficking, immune response, and inflammation, as well as recognition of extracellular matrix.

Lectins are a diverse group of carbohydrate-binding proteins that are found within and associated with organisms from all kingdoms of life. Several different classes of plant lectins serve a diverse array of functions. The most prominent of these include participation in plant defense against predators and pathogens and involvement in symbiotic interactions between host plants and symbiotic microbes, including *mycorrhizal fungi* and nitrogen-fixing rhizobia.

Purified lectins are important in a clinical setting because they are used for blood typing . Some of the glycolipids and glycoproteins on an individual's red blood cells can be identified by lectins.

• A lectin from Dolichos biflorus is used to identify cells that belong to the A1 blood group.

• A lectin from Ulex europaeus is used to identify the H blood group antigen.

• A lectin from Vicia graminea is used to identify the N blood group antigen.

• A lectin from " Coconut milk" is used to identify Theros antigen.

• A lectin from " Dorex is used to identify R antigen.

Lectin may cause leptin resistance, affecting its functions (signal have high levels of leptin and several effects gathering to protect from lipid overload), as indicated by studies on effects of single nucleotide polymorphisms on the function of leptin and the leptin receptor. Such leptin resistance may translate into diseases, notably it could be responsible for obesity in humans who have high levels of leptin.

In neuroscience, the anterograde labeling method is used to trace the path of efferent axons with PHA-L, a lectin from the kidney bean. A lectin (BanLec) from bananas inhibits HIV-1 in vitro. Microvirin (MVN), a recently isolated lectin from the cyanobacterium *Microcystis aeruginosa* PCC7806, shares 33% identity with the potent anti-human immunodeficiency virus (HIV) protein cyanovirin-N (CV-N) isolated from *Nostoc ellipsosporum*, and both lectins bind to similar carbohydrate structures [64,71]. BanLec is a jacalin-related lectin isolated from the fruit of bananas, *Musa acuminata*. This lectin binds to high mannose carbohydrate structures, including those found on viruses containing glycosylated envelope proteins such as human immunodeficiency virus type-1 (HIV-1). Legumin, albumin-2, defensin and albumin-1 were previously identified as contributing to the increased sulfur amino acid content in the mutant line, on the basis of similarity to proteins from other legumes [72].

Use in studying carbohydrate recognition by proteins

Lectins from legume plants, such as PHA or concanavalin A, have been widely used as model systems to understand the molecular basis of how proteins recognize carbohydrates, because they are relatively easy to obtain and have a wide variety of sugar specificities. The many crystal structures of legume lectins have led to a detailed insight of the atomic interactions between carbohydrates and proteins. Concanavalin A and other commercially available lectins have been widely used in affinity chromatography for purifying glyco proteins. In general, proteins may be characterized with respect to glycoforms and carbohydrate structure by means of affinity chromatography, blotting, affinity electrophoresis and affinity immunoelectrophoreis with lectins. Identification of sulfur-rich proteins whose levels are elevated in seed lacking *phaseolin* and phytohemagglutinin and sulfur metabolic genes may assist the improvement of protein quality [73].

Use in biochemical warfare

One example of the powerful biological attributes of lectins is the biochemical warfare agent ricin. The protein ricin is isolated from seeds of the castor oil plant and comprises two protein domains. Abrin from the jequirity pea is similar:

- One domain is a lectin that binds cell surface galactosyl residues and enables the protein to enter cells

- The second domain is an N-glycosidase that cleaves nucleobases from ribosomal RNA, resulting in inhibition of protein synthesis and cell death.

Digestion and immune distress

Foods with high concentrations of lectins, such as beans, cereal grains, seeds, and nuts, may be harmful if consumed in excess in uncooked or improperly cooked form. Adverse effects may include nutritional deficiencies, and immune (allergic) reactions. Possibly, most effects of lectins are due to gastrointestinal distress through interaction of the lectins with the gut epithelial cells. In study has suggested that the mechanism of lectin damage may occur by interfering with the repair of already-damaged epithelial cells. Recent research indicates that lectin had no antifungal activity. It did not stimulate nitric oxide production by murine peritoneal macrophages. Chemical modification results indicated that tryptophan was crucial for the hemagglutinating activity of the lectin [74-76].

The mannose receptor (MR):

It is a Group VI C-type lectin. Its expression was originally thought to be restricted to mammalian tissue macrophages but it is now known to be expressed on lymphatic and hepatic

epithelium, kidney mesangial cells, tracheal smooth muscle cells and retinal pigment epithelium [77]. The MR binds a broad array of microorganisms. The receptor recognizes mannose, fucose or N-acetylglucosamine sugar residues on the surfaces of these microorganisms [78]. The MR has been implicated in the phagocytic uptake of pathogens, but there are limited examples actually demonstrating MR-dependent phagocytosis. The first suggestion that the MR was a phagocytic receptor was based on the mannan-inhibitable uptake of zymosan by mouse peritoneal macrophages. The MR has also been implicated in the phagocytic uptake of apoptotic cells in COPD [79].

Dectin-1: is classified as a Group V non-classical C-type lectin. It was as a receptor for β-glucans. By way of its β-glucan specificity, Dectin-1 can recognize a number of fungal species.

DC-SIGN (CD209): DC-SIGN is often described as a phagocytic receptor. This is conceivable given its interactions with pathogens and the presence of internalisation motifs (di-leucine motif, tri-acidic cluster, ITAM motif) in its cytoplasmic tail [80]. However, to date, the evidence for the phagocytic potential of DC-SIGN has been indirect and still needs to be demonstrated conclusively. phagocytic potential of C-type lectins Phagocytosis is phylogenetically conserved in mammals and has evolved into a remarkably complex process. This process control extracellular pathogens, and this activity is mediated by several pattern recognition receptors (PRRs), including a number of C-type lectins.

The C-type lectin superfamily is a large group of proteins which have one or more C-type lectin-like domains (CTLDs). The super- family is divided into 17 groups based on their phylogeny and domain organization [81].

SUMMARY

lectins can be used to differentiate malignant tumors from benign cells and their degree of glycosylation, which is associated with metastasis. It has also been demonstrated that lectins inhibit cell proliferation and have ytotoxic effects on human tumor cells [82]. lectins have received more attention from cancer biologists due to their remarkable anti-

tumor properties compared to the other lectin families. lectins ConA, ConBr, and CFL are all structurally related and induce apoptosis in the MCF-7 cell line. Lectins reduce both proliferation and viability of leukemic cells. The MTT-based assay and total nucleic acid content (NAC) measurements show that ConA and ConBr lectins have cytotoxic effects in leukemic cells. Lectins(Con A and Con Br) induce internucleosomal DNA fragmentation and alter mitochondrial transmembrane potential in leukemic cells. ConA and ConBr induce apoptosis in leukemic cells by triggering an intrinsic mitochondrial pathway. Lectins increase ROS (Reactve Oxygen Species) in leukemic cells [83].

The cytotoxicity exhibited by the lectins ConA and ConBr on tumor cells was caused mainly by induction of cell death via apoptosis, but also by necrosis when they are at higher concentrations. Thus, the cytotoxic agent may induce either apoptosis or necrosis depending on the concentrations and time of contact with the substance. Generally, apoptosis induction in tumor cells is a beneficial effect for chemotherapy treatment of cancer. The lectins may promote apoptosis via two mechanisms. One possibility is by interacting with the cell surface, being endocytosed, and then reaching the mitochondria. This possibility would occur directly through the intrinsic pathway, as with ConA in some cell lines and other lectins such as WGA. A second possibility is by binding to glycosylated portions of death receptors and then leading to its activation and apoptotic signal transduction through the extrinsic pathway [84].

CONCLUSION

Lectins are believed to act as modulations of cell substratum interactions and to be essential for the normal differentiation and growth of all multicellular animals. They are capable of inducing cell proliferation [85] cell arrest, or apoptosis (physiological cell death) and have been implicated in organ morphogenesis, tumor cell metastasis, leukocyte trafficking, skin test for immunological assessment response, and inflammation, as well as recognition of

extracellular   matrix in an addition to well establish effect of PHA on mitotic stimulation, and also based on the current   literature, the advantages of PHA agent would indicate the potential   sources   for   developing   novel pharmaceutical and phtomedicine preparation.

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