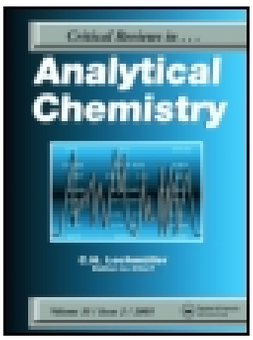

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ANALYTICAL APPLICATION OF LECTINS

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Abstract

This review is devoted to the analytical application of carbohydrate-binding proteins called lectins. The nature of lectins and the regularities of their specificity with respect to simple sugars and complex carbohydrate-containing biomolecules are discussed. The main areas of the modern analytical application of lectins are described. Lectin-affinity chromatography, histo- and cytochemical approaches, lectin blotting, microarray, and biosensor technologies as well as microplate analysis are considered in detail. Data on the use of lectins for the detection of cells and microorganisms as well as the study of protein glycosylation are summarized. The large potential of lectins as components of analytical systems used for the identification of glycans and the characteristics of their structure are substantiated.

Keywords: lectins; carbohydrates; lectin–carbohydrate interactions; analytical methods

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1. Introduction

Carbohydrates are part of every cell of any living organism; most proteins and lipids in the human body are glycosylated. Studies of the glycosylation of intracellular and membrane proteins as well as of proteins in biological fluids have revealed many links between glycosylation and various pathologies (Gornik et al., 2012; Lemjabbar-Alaoui et al., 2015; Pu and Yu, 2014; Vaidyanathan and Wells, 2014; Zhu et al., 2014). All this explains the active development of glycomics—a new research field studying the structures of carbohydrate and glycoconjugates (Hirabayashi, 2014).

Glycans are characterized by huge structural diversity and currently studied much less than proteins and nucleic acids. Therefore, their investigation is a complicated task that determines the ability to develop effective tools for detecting carbohydrate structures and their changes under various physiological and pathological conditions (Gabius et al., 2002).

The detection and identification of glycans can usually be carried out using high-performance liquid chromatography and mass spectrometry (Haab, 2012). These methods, ensuring reliable glycan determination, have several significant drawbacks: complicated sample preparation, the need for expensive equipment and qualified personnel, and the complexity of the simultaneous analysis of numerous samples carried out in diagnostic studies. In this regard, the use of lectins characterized by high specificity and affinity toward glycans is most promising for their detection and the study of biomolecules and cells' glycosylation. Methods based on carbohydrate–lectin interactions allow researchers to solve such problems as separating and purifying carbohydrate-containing biomolecules, studying the carbohydrate component of biomolecules, and detecting pathogens. These opportunities are discussed in several recent

reviews (Brooks, 2017; Dan et al., 2016; Hirabayashi, 2014; Hirabayashi et al., 2011; Solis et al., 2015; Van Breedam et al., 2014).

This review is devoted to the use of lectins for the characterization of carbohydrate-containing structures and relevant biological processes and contains information on the main analytical methods implemented based on lectins.

2. History of lectin research

As a compound agglutinating erythrocytes, a lectin from castor bean seeds *(Ricinus communis)* was for the first time isolated more than 80 years ago (Sumner and Howell, 1936). The researchers demonstrated that the interaction of agglutinins with biological receptors did not have a catalytic or immune nature. Therefore, carbohydrate-specific antibodies or enzymes of carbohydrate metabolism that cause structural changes in carbohydrate molecules as a result of the interaction with them do not belong to the lectin group. The term “lectin” is derived from the Latin word “legere,” which means “to collect.” Lectins belong to a group of proteins capable of selective nonimmune binding with carbohydrate-containing compounds.

The first purified hemagglutinin—concanavalin A (Con A) from jack beans (*Canavalia ensiformis*)—was obtained in 1919 (Sharon and Lis, 2004). The ability of lectins to differentiate blood groups was discovered in the 1940s. Lectins specifically agglutinating erythrocytes were used to detect antigens of the ABO blood group system. Many lectins were isolated and purified after the development of the affinity chromatography method (Nascimento et al., 2012). The ability of lectins to agglutinate malignant cells was discovered in 1960s. Aub et al. (1963) studied surfaces of tumor cells and showed that the lipase from wheat seeds caused adhesion of

only tumor cells. When the enzyme was completely inactivated by heating, the aggregation effect persisted, indicating the presence of a functional compound that mediated such aggregation. This compound was a wheat germ agglutinin (from *Triticum vulgaris*), WGA, isolated and characterized by Burger and Goldberg (1967). The authors showed that WGA-mediated agglutination was inhibited by *N*-acetyl-*D*-glucosamine (GlcNAc), which competed with hemagglutinin to bind to the cell surface. Subsequently, the differentiation of tumor cells and their subpopulations was carried out using other lectins (Gillespie et al., 1993).

The development of affinity chromatography and the emergence of recombinant technologies promoted intensive studies of the isolation and purification of numerous lectins not only from plant sources but also from animal tissues, microorganisms, and viruses (Lam and Ng, 2011; Nascimento et al., 2012). Physicochemical properties of many lectins have been studied; their amino acid sequences were deciphered and spatial structures of the molecules were determined (Sharon and Lis, 2004).

Over the past decade, great progress has been made in lectinology; many new lectin-based analytical approaches have been developed that open up broad prospects for further studies of carbohydrates' properties and functions and the development of effective systems for their detection. Several studies have published results that describe the use of lectins in the study of glycoconjugates in solution and on the surface of cells, the detection of lectin-reactive structures in biological objects, the fractionation and purification of glycoconjugates, the detection of various pathogens, and so on (Brooks, 2017; Dan et al., 2016; Hirabayashi, 2014; Hirabayashi et al., 2011; Van Breedam et al., 2014). The main milestones in the history of lectin research are presented in Figure 1.

3. Nature and specificity of lectins

Lectins are a group of proteins of nonimmune origin that are present in microorganisms, plants, and animals and specifically and reversibly bind carbohydrates and the carbohydrate determinants of biopolymers without changing their structures (Lakhtin et al., 2011; Zeng et al., 2012). Lectins play an important role in many processes associated with the recognition and interaction of cells with various objects, the synthesis and transport of proteins, photosynthesis, the regulation of cell division, fertilization, congenital immunity, and so on (De Schutter and Van Damme, 2015; Feizi and Haltiwanger, 2015).

Until recently, lectins were classified by their reactivity to monosaccharides. According to this classification, the family of carbohydrate-binding proteins was divided into mannose-binding, galactose-binding, chitin-binding, and other lectins. This classification was recognized as artificial because it did not consider the structural features of protein molecules. With the development of genomic/transcriptomic analysis and the ability to decipher the structure of polypeptide chains in lectin molecules, another classification was adopted based on the similarity of amino acid sequences in the molecules of these proteins and the structure of their carbohydrate recognition domains (CRD). Each CRD is characterized by a unique amino acid sequence, a typical fold structure of the polypeptide, and a binding site structure. Accordingly, all plant lectins are divided into 12 families: the *Agaricus bisporus* homolog, amaranthin domain, homolog of class V chitinases, cyanovirin domain, *Euonymus europaeus* lectin domain,

Galanthus nivalis agglutinin domain, hevein domain, jacalin domain, legume domain, Lys M domain, nictaba-like domain, and ricin-B domain (Van Damme, 2014). Different CRDs can interact with similar carbohydrate structures; hence, the specificity of lectins is not determined only by the carbohydrate-recognizing domain (Van Damme et al., 2008).

Most of the known lectins are characterized by their multimerity; their molecules consist of two or more covalently linked subunits (Lakhtin, 1994). In this case, the subunits can have either identical (as in Con A) or different structures (as with *Phaseolus vulgaris* agglutinin, PHA). The presence of several subunits determines the possibility of the agglutination of cells by lectins.

The most important aspect of lectins in determining their quality as an analytical tool is their specificity. The specificity of the carbohydrate–lectin reaction is determined by the presence of certain carbohydrate residues in glycan molecules. Lectins can interact with both mono- and oligosaccharides and with carbohydrate residues in complex biomolecules, such as polysaccharides, glycoproteins, and glycolipids. Compared to antibodies, lectins are less complementary to recognizable molecules. According to the literature, typical K_a reactions of carbohydrate–lectin are 10^3–10^7 M^{-1} (Gabius et al., 2011; Lebed et al., 2006). However, the affinity of the polyvalent interaction of lectins with polysaccharides becomes comparable with the affinity of the immune reactions. Unlike antibodies that have an affinity for a unique antigenic determinant, lectins do not have such high specificity. The carbohydrate–lectin interaction depends on many factors, such as lectin valence, the structure of binding sites, their spatial arrangement, and so on (Lakhtin, 1994).

Lectins are specific to certain carbohydrate structures. Thus, some lectins can interact only with mannose (Man) or glucose (Glc) residues (for example, Con A and *Lens culinaris* agglutinin, LCA) and others only with galactose (Gal) (for example, peanut agglutinin, PNA and *Ricinus communis* agglutinin, RCA). There are lectins specific to fucose (for example, *Ulex europaeus* agglutinin, UEA; *Aleuria aurantia* lectin, AAL; and *Ralstonia solanacearum* lectin, RSL), sialic acid (for example, *Maackia amurensis* lectin, MAL), and other monosaccharides. The characteristics of the specificity of many lectins are presented, in particular, in the study by Kobayashi et al. (2014). Some lectins can interact only with terminal carbohydrate residues in complex biomolecules, while others can bind sugar residues within one carbohydrate chain. Several lectins not only are specific to the definite anomeric form of sugar molecules (α- or β-anomer) but also require a strict sequence of carbohydrate residues. Data on the main characteristics of lectins, their specificity, and their belonging to different families are presented in Figure 2 and Table 1.

The multivalence of carbohydrate–lectin interactions increases their selectivity. While the affinity of the lectin–monosaccharide interaction is relatively low, the specificity of lectins to branched carbohydrates increases. Because of the multi-interaction mechanism of additional binding in the subsites of carbohydrate molecules or because of the length of binding sites and the polyvalence of lectin subunits, the selectivity of carbohydrate–lectin binding can increase by several orders of magnitude (Rini, 1995; Weis and Drickamer, 1996). In this case, one monosaccharide residue (usually terminal) interacts with the main lectin binding site, and other monosaccharides interact with secondary sites along the carbohydrate chain. Such selectivity increase is observed, in particular, during the interaction of Glc/Man-specific *Lathyrus ochrus* lectin, LOL I, with Man-containing oligosaccharides (Bourne et al., 1992) and cholera toxin (CT) with GM1-gangliosides containing sialic acid and galactose terminal residues (Merritt et al., 1994). Subunit polyvalence occurs if several lectin subunits either bind to carbohydrate residues on one branched carbohydrate chain, as in the asialoglycoprotein receptor (Rice et al., 1990), or interact with binding sites on several carbohydrate chains, as in trimeric mannose-binding protein or pentameric CT (Merritt et al., 1994).

Although maximum lectin selectivity is achieved through multivalent interactions, their specificity to monosaccharides, including those in complex branched carbohydrate molecules, remains a crucial condition for lectins' recognition of carbohydrates. Thus, Glc/Man-specific lectins from legume family plants (for example, LOL I and Con A) bind complex carbohydrates containing Glc and Man; the presence of a galactose residue, or its amino acetylated derivative in the carbohydrate chain, is necessary for the binding of Gal-specific lectins (for example, *Erythrina corallodendron* lectin, ECoRL) (Shaanan et al., 1991). Gal and Glc/Man are epimers

with different stereochemistry at the C-4 position. Likewise, C-type lectins (mannose-binding protein and asialoglycoprotein receptor) specific to Man and Gal interact with the corresponding terminal monosaccharides in complex carbohydrate molecules (Drickamer, 1992). However, the specificity of the primary complexation with lectins and overall specificity may significantly differ. In the case of a CT, galactose and sialic acid are of crucial importance for binding with GM1 (Merritt et al., 1994), whereas the monosaccharide specificity of CT is demonstrated only in relation to galactose (Merritt et al., 1994). Likewise, galactose plays a key role in the interaction of *Griffonia simplicifolia* lectin (GSL) with the Lewis b blood group oligosaccharide, but galactose as a monosaccharide does not recognize this lectin (Rini, 1995).

Despite the general nature of the key interactions responsible for the recognition of carbohydrates, the stereochemistry of binding sites for each complex is unique enough to discriminate ligands (Rini, 1995; Weis and Drickamer, 1996). A detailed consideration of the Gal and Glc/Man discrimination by lectins reveals that the distribution of the atoms participating in the formation of hydrogen bonds in the main binding site considers the ligand-dependent stereochemical features of the hydrogen bond in the 4-OH region. In combination with the preferential arrangement of aromatic residues, these features can play a key role in primary specificity (Kolatkar and Weis, 1996; Rini, 1995; Weis and Drickamer, 1996; Elgavish and Shaanan, 1997).

The main areas of the modern analytical application of lectins classified by types of methods are considered below.

4. Lectin-based analytical methods

4.1. Traditional analytical methods

Analytical methods based on lectin–carbohydrate interactions can be conventionally divided into traditional and modern ones. The semi-quantitative method of hemagglutination inhibition, based on a lectin-mediated cell agglutination process, belongs to the traditional methods (Sano and Ogawa, 2014). This method was modernized by using a radioactively labeled lectin, which resulted in an increase in the method's sensitivity (Duk et al., 1982). Conventional methods also include precipitation analysis originally developed for the detection of antigen–antibody complexes. This approach was based on the inhibition of lectin precipitation of high molecular weight glycoconjugates by mono- and oligosaccharides and had serious limitations associated with the large quantities of reagents required for analysis (Wu et al., 1997). However, high accuracy has contributed to this method's practical use for a long time. In the 1990s, the above-described approaches were replaced by solid-phase analysis, described below.

4.2. Lectin affinity chromatography

Currently, lectins are widely used as sorbents, and lectin affinity chromatography is a common method for the isolation, fractionation, and purification of carbohydrate-containing structures. Affinity chromatography using lectins was first developed by Donnelly and Goldstein (1970). Because the interaction of lectins with carbohydrates has a non-covalent character and is characterized by a frankly low affinity, glycans can be competitively displaced from the specific complex by a competitor compound. This provides additional opportunities in the implementation of lectin affinity chromatography. Because the specificity of some lectins may be low, two or more glycans may bind to a carrier during affinity chromatography (Durham and

Regnier, 2006). For their further fractionation, a serial lectin affinity chromatography (SLAC) involving multiple elution to provide a better resolution of glycans can be used as a variation of this approach.

Thus, the combination of Con A (specific to mannose) and lectin from *Artocarpus altilis* (specific to GalNAc in *O*-glycosylated and Man-containing sites of *N*-glycans) was used to isolate *O*-glycosylated peptides in the study of O-glycosylation sites of human serum proteins carried out by Durham and Regnier (2006). Sumi et al. (1999) used lectin affinity chromatography to differentiate between carcinoma and prostatic hyperplasia through differences in the carbohydrate structures of the prostate-specific antigen (PSAg). For this purpose, a consecutive series of columns with immobilized Con A, PSA, WGA, phytohemagglutinin E4 (PhHA-E4), and phytohemagglutinin L4 (PhHA-L4) was used. Quantitative analysis of the fractions showed that, in the case of prostate carcinoma, the level of the multi-antenna complex with branched GlcNAc (1→4) mannose was significantly increased.

Alpha-fetoprotein (AFP) is used as a serum marker for the diagnosis of hepatocellular carcinoma. However, the specificity of this approach can be enhanced by the detection of glycosylated forms of this protein. Debruyne and Delanghe (2008) increased the specificity of the differentiation of benign and malignant neoplasms up to 95%, which is significantly greater than when determining the total AFP. Lobo et al. (2017) applied lectin affinity chromatography on a column with sepharose-immobilized *Artocarpus incisa* lectin to study the correlation between changes in protein glycosylation and the progression of breast cancer.

4.3. Lectin histochemistry and cytochemistry

The carbohydrate-binding properties of lectins were first used for histological staining of samples in the 1980s. Alroy et al. (1987) detected biotinylated lectins bound to glycoproteins in the samples and then to avidin-peroxidase conjugates with the help of peroxidase's colorimetric substrate 3.3-diaminobenzidine. The history, state of the art, and prospects of lectin histochemistry development are detailed in reviews by Brooks (2017) and Manning (2017). For histological analyses, lectins conjugated with fluorescent (e.g., fluorescein isothiocyanate) and enzymatic (e.g., horseradish peroxidase, HRP) labels, haptens (biotin, digoxigenin, etc.), and gold nanoparticles and visualized by fluorescence measurement, light, or electron microscopy are used (Roth, 2011; Nakajima, 2009). Leal et al. (2012) visualized *Aspergillus* structures in histological samples of brain and lung tissue using HRP-conjugated WGA and Con A. Atabaki Pasdar et al. (2013) used a panel of eight conjugated lectins to detect changes in the glycosylation of chorionadenoma cells. Kostrominova (2011) used a fluorescently labeled WGA to visualize skeletal muscle fibers and connective tissue. Seco-Rovira et al. (2013) applied lectins to carry out the histochemical determination of apoptotic spermatocytes of Syrian hamster (*Mesocricetus auratus*) spermatids. It has been shown that the amount of glycoconjugate residues in *β*1-Gal, 3-GalNAc*α*1, *α*-*D*-mannose, GalNAc, and *L*-fucose correlates with the level of apoptosis of the germ cells.

Because the glycosylation of proteins finishes in the Golgi apparatus, this intracellular structure contains many different glycoproteins, being a specific target for lectins (Moremen et al., 2012). Moremen et al. (2012) suggested a method based on the specific staining of trans-Golgi in fixed cells by WGA conjugates. The *Griffonia simplicifolia* lectin II, demonstrating high specificity to the terminal α- and β-*N*-acetyl-*D*-glucosaminyl (GlcNAc) residues of

glycoproteins, can be used to stain trans-Golgi-intermediates, and *Helix pomatia* agglutinin can specifically interact with α-*N*-acetylgalactosaminyl residues (Moremen et al., 2012).

Labeled lectins are also effective in the study of membrane transport. For example, the redistribution of glycoproteins, which disrupts the "endoplasmic reticulum–Golgi apparatus," transport initiated by guanosine-5'-O-(3-thio)-triphosphate was studied with the help of *Galanthus nivalis* agglutinin (Morgan et al., 2013). Fluorescently labeled lectins can also be used in sorting cells in the flow cytometry method (Onuma et al., 2013).

Therefore, lectins are a very important tool for the detection of glycoproteins in tissues, individual cells, or organelles as well as in interactions involving glycans in the intercellular space. Thus, WGA allows effective tracking of the transsynaptic chain in anatomical studies (Yoshihara et al., 1999). Yoshihara et al. (1999) were the first to use the WGA–cDNA conjugate under the control of neuron-specific promoter elements to visualize nerve structures that are anatomically and functionally related to each other. This technology has demonstrated high accuracy and reproducibility, and the WGA-transgene has become an excellent genetic tool for the visualization of neural structures (Ohmoto et al., 2010; Walling et al., 2012; Yoshihara et al., 1999).

4.4. Lectin blotting

The same principles as in immunoblotting underlie the lectin blotting approach. After electrophoretic separation, the proteins are transferred to the nitrocellulose membrane, incubated first with biotinylated lectins and then with the streptavidin–enzyme conjugate (see Figure 3). The formed complexes are detected through the appropriate substrate (Morgan et al., 2013).

Lectin blotting is currently widely used in various investigations (Norton et al., 2016; Vainauskas et al., 2016). The joint application of lectin-histochemical studies and lectin blotting allows for a better characterization of changes in the glycosylation of cells during the development of primary tumor and metastatic processes. The combination of mass spectrometry and lectin blotting can help in determining the type of alliterated glycoproteins. Rambaruth et al. cut gel-stained bands formed during the lectin blotting of the lysates of breast cancer cells and analyzed them by mass spectrometry (Rambaruth et al., 2012). They demonstrated that heat shock protein 27 (Hsp27), *D*-like HnRNP, HnRNP A2/B1, enolase 1 (ENO1), and several other proteins showed an increased level of regulation in the metastatic cells of MCF7 and T47D, and that the established involvement of Hsp27 and ENO1 glycoproteins in apoptosis and cell migration can contribute to metastasis.

Ueda et al. (2013) found that cells in the G1 phase are more susceptible to the influenza virus than cells in the S/G2/M phase and investigated the molecular basis of this process by analyzing sialic acid on the surface of the cell membranes, which is a target of the influenza virus. The digoxigenin-labeled MAL and SNA specific to sialic acid were used to detect sialic acid molecules containing α2-3 and α2-6 bonds via lectin blotting. The researchers found that the level of sialic acid with both forms of bonds was increased in G1-phase cells.

Although lectin blotting is not as reliable a method for glycoprotein detection as biosensor technology (discussed below), it has good prospects because of the simplicity of implementation and the accessibility of reagents.

4.5. Lectin microarrays

Lectin microarrays have recently been used as a promising tool for glycan profiling (Hirabayashi et al., 2015; Syed et al., 2016). In this technology, several lectins with known specificity are immobilized as microdots on a solid surface (for example, glass). The carbohydrate residues of the analyzed compounds interact with the corresponding lectins (Figure 4). The binding of glycans with lectins allows for a quick determination of their specificity and the registration of even very weak interactions with very high accuracy. The detection of such interactions can be carried out using one of several approaches—in particular, confocal fluorescence and evanescent-field activated fluorescence (Hirabayashi et al., 2013; Kuno et al., 2005). A method such as bimolecular fluorescence quenching and recovery detection does not require the preliminary labeling of target carbohydrates (Koshi et al., 2006).

Lectin microarrays, first described in 2005, are currently in demand as an analytical tool (Hirabayashi et al., 2015). A variety of microarrays have been recently proposed for the detection of individual objects—germ (Xin et al., 2014) and stem (Nand et al., 2014) cells, bacteria (Yasuda et al., 2014), and fungi (Shibazaki and Gonoi, 2014)—as well as to diagnose pathological processes (Inoue et al., 2013; Nakajima et al., 2015).

Lectin microarrays can serve as a powerful tool in the search for markers of cancer and other pathologies. It should be noted that many known biological markers have a glycoprotein structure. Methods for pathology detection based on the determination of the protein component of a marker are often clinically ineffective. Li et al. (2011) used lectin microarray to study differences in the glycosylation of PSAg molecules in healthy people and oncological patients having aggressive as well as nonaggressive cancer forms and metastatic processes. Lectins interacting with PSAg were used to study the glycosylation of samples using microplate analysis. According to the obtained results, the pairs PSAg–SNA and PSAg–jacalin were recognized as effective means to discriminate aggressive cancer forms. The new fetuin A biomarker, effective for predicting the development of diabetic nephropathy, was identified by analyzing patients' urine samples with the help of lectin microarray technology (Inoue et al., 2013).

Lectin microarray technology has found wide application in the distinguishing of cells. Tateno et al. (2011) developed a microarray with 96 immobilized lectins for a complete analysis of the glycan composition of human-induced pluripotent stem cells (iPSCs) obtained from four types of somatic cells (SCs). Glycan profiles of iPSC were compared with the profiles of nine types of human embryonic stem cells (ESCs). As a result, differentiated SCs and undifferentiated iPSC/ESCs were identified as two separate groups. This indicated, first, the difference between the glycan profiles of somatic and embryonic stem cells and, second, that in the induction of pluripotency, SCs acquire glycans characteristic of embryonic stem cells (Tateno et al., 2011). In addition, the authors determined that the α1-2Fuc-specific recombinant lectin from *Burkholderia cenocepacia*, rBC2LCN, is the best tool for distinguishing between undifferentiated iPSC/ESCs

and differentiated SCs and recombinant MOA for the detection of contamination by murine embryonic fibroblasts in human iPSCs.

A similar study carried out by Toyoda et al. (2011) demonstrated that *Euonymus europaeus* lectin (EEL), MAL, and phytohemagglutinin L (PhHA-L) allow researchers to distinguish between undifferentiated and differentiated human embryonic stem cells. Xin et al. 2014) developed a lectin microarray with 91 immobilized lectins for the complex profiling of the glycocalyx of spermatozoa.

Very important results concerning the mechanisms of masking and releasing the human immunodeficiency virus from the host cell were obtained by Krishnamoorthy et al. (2009). The authors compared the glycosylation of cell membranes and microvesicles (MV) in uninfected H9 and HIV-1 T cells of strain CL.4. It was found that, unlike blood group antigens expressed on the surface of uninfected cells, H9 HIV and MB cells have similar carbohydrate structures containing large quantities of mannose, complex *N*-linked glycans, *N*-acetyllactosamine, sialic acid, and fucosylated epitopes. This suggests that that the immunodeficiency virus can be masked as immunomodulating microvesicles to “deceive” the immune system and leave the host cell through a mechanism similar to the exit mechanism of MV (Krishnamoorthy et al., 2009). Sharma et al. (2016) showed that bovine lactoferrin (BLF) is of vital importance at an early stage of lactation, having antimicrobial and antiadhesive properties, antitumor activity, and so on. O’Riordan et al. (2014) demonstrated that the glycan components of BLF play an important role in its biological functions. The authors used lectin microarray technology to study changes in the glycosylation of bovine lactoferrin at different stages of lactation and compared the carbohydrate composition of BLF in colostrum and transitional and mature cow milk. This investigation

showed the great potential of lectin microarray technology in monitoring of protein glycosylation.

Glycan microarrays were first developed in 2002 (Drickamer, 1992; Park and Shin, 2002). There, glycans are immobilized in a solid phase (de Boer et al., 2008; Katrlik et al., 2010; Xia et al., 2005). Glycan microchips are, for example, an effective means of analyzing the specificity of influenza virus receptors, allowing for rapid risk assessment of new human viruses (Blixt et al., 2004). Lectin and glycan microarrays are now commercially available. Lectin microarrays are produced by companies such as Procognia, GlycoTechnica, Plexera, and Raybiotech, among others. Some microarrays are reusable (for example, the Lectin Array Chip Kit from Plexera). Glycan array analytical systems (molecular biotechnology) contain arrays of more than 20 glycans. Glycan Array 100, containing 100 of the most common glycans, is produced by Raybiotech. Zhang et al. (2016) evaluated the utility of a lectin microarray for assessing protein glycans using commercial lectin chips. With the use of 45 immobilized lectins specific to distinct glycan structures, the lectin binding patterns of a panel of 15 therapeutic proteins, including eight monoclonal antibodies, were determined. Lectin-based microarray was found to be highly sensitive to the carbohydrate structures of galactose and sialic acid. The authors demonstrated that lectin microarray technology can be used to screen glycan patterns of therapeutic glycoproteins.

4.6. Lectin biosensors

Lectin-based biosensors are widely described in the literature (Akiba and Anzai, 2016; Belicky et al., 2016; Chen et al., 2017; Klukova et al., 2016). One of the first fluorescent

biosensors based on Con A was developed to detect various carbohydrates (Hamachi et al., 2000). Con A was modified by attaching a photoaffinity labeling reagent to the carbohydrate binding pocket. The principle of detection was based on the change in the maximum and intensity of fluorescence upon the interaction of specific saccharides with Con A. It should be noted that the modification of lectin molecules can lead to a change in their binding properties. Therefore, numerous attempts have recently been made to develop biosensor systems based on unlabeled lectins.

An electrochemical biosensor based on lectin–carbohydrate interactions was first proposed by Dai et al. (2006). PNA was immobilized on a gold substrate coated with a monolayer having a self-assembling structure. The competitive interaction between the nanocrystal-labeled carbohydrate and the analyte was monitored by amperometric inversion voltammetry. The authors found that the voltammetric peak current decreases with the increasing affinity of glycan binding. The developed method was characterized by high sensitivity: the detection limit of the cancer-associated T antigen (*β-D*-Gal-[1-3]-*D*-GalNAc disaccharide) was 0.1 μM.

Many label-free technologies, including surface plasmon resonance (SPR), quartz crystal microbalance (QCM), and electrochemical impedance spectroscopy (EIS), can be successfully used to detect and profile glycans (La Belle et al., 2007). SPR is widely used for the real-time detection of molecular interactions and the study of surface phenomena. Upon lectin–carbohydrate binding on a surface of the sensor chip, the refractive index of the medium and, consequently, the angle of reflection of the incident light changes (Figure 5). The concentration of the analyzed glycan can be determined by estimating the change in the SPR angle, which is

proportional to the mass of the bound analyte. The use of QCM is based on a change in the resonance frequency of a quartz crystal as a result of mass accumulation upon carbohydrate–lectin interactions (Ayela et al., 2007). EIS is based on a change in the amplitude of an alternating current and the corresponding interfacial characteristic—for example, resistance—upon carbohydrate–lectin interactions. The first label-free lectin-based EIS biosensor was developed by LaBelle et al. (2007). The binding of Galα1-3GalNAc disaccharide, asialofetuin, and fetuin with PNA and SNA covalently immobilized on the surface of Cu/Ni/Au-printed electrodes was monitored by the change in impedance. The detection limits of these compounds were very low (150 fM).

Many signal amplification methods have been developed to increase the sensitivity of biosensors. One of the strategies is the use of nanoparticles. Self-assembled monolayers are used to control the properties' interface and facilitate the deposition of nanoparticles, thus increasing their biomolecule-loading capacity (Bertok et al., 2013). Bertok et al. (2013) developed an ultrasensitive lectin-biosensor system to detect sialic acids using this enhancement approach. A flat gold electrode was immersed in a solution of aminoalkanethiol to form a monolayer for the binding of gold nanoparticles. A second monolayer was formed by 11-mercaptodecanoic acid and 6-mercaptohexanol with covalently attached sialic acid—specific SNA. The authors detected the interaction of SNA with fetuin and asialofetuin containing sialic acid by increasing the resistance at the electrode. The detection limit of 1 aM was significantly lower than that of other analytical systems (Bertok et al., 2013).

Zhang et al. (2010) developed a biosensor based on SNA and Man-specific Con A to compare the expression of sialic acid and mannose in normal and cancer cells of the lungs, liver, and prostate. They found the expression levels of mannose are comparable in normal and cancer cells, while the level of sialic acid is increased in tumor cells. The SNA-based biosensor allowed for not only the quantitative detection of cancer cells but also for the estimation of the content of sialic acid on the cell surface, which is very important in assessing cancer progression. Chen et al. (2017) developed a nanostructured biosensor for the detection of tear glucose levels using a fluorescence resonance energy transfer (FRET) quenching mechanism. CdSe/ZnS quantum dots (QDs) were used as a donor, and dextran-binding malachite green (MG-dextran) was conjugated to Con A; a lectin with specific affinity to glucose was used as an acceptor. In the presence of glucose, the emission of QDs was quenched through the FRET mechanism and restored by displacing the dextran from Con A. The detected glucose concentration was converted to fluorescence spectra with a high signal-to-noise ratio and calibrated image pixel value. The linear range of detected Glc was 0.03–3 mmol/L, which allows the estimation of tear glucose levels for both diabetics and healthy subjects. Klukova et al. (2016) developed a label-free ultrasensitive impedimetric biosensor for glycoprotein determination with a detection limit of 1 aM. A lectin was directly immobilized on a graphene-oxide-based interface without any polymer modifier.

Bertok et al. (2013) developed a label-free lectin-based impedimetric biosensor for the detection of the sialylated glycoproteins fetuin and asialofetuin. A lectin from *Sambucus nigra* I was covalently immobilized on a mixed self-assembled monolayer. The biosensor exhibits a linear range that spans seven orders of magnitude for both glycoproteins, with detection limits as low as 0.33 fM for fetuin and 0.54 fM for asialofetuin. Moreover, the developed biosensor was

capable of quantitatively detecting changes in the fraction of sialic acid on glycoproteins and could potentially be applied for the diagnosis of various diseases associated with the aberrant glycosylation of protein biomarkers. Ma et al. (2015) developed a label-free lectin biosensor for the quantitative measurement of the interaction between lipopolysaccharide (LPS) on Gram-negative bacteria and immobilized Con A. Binding detection was carried out through orthogonal quartz crystal microbalance and electrochemical readouts. The developed biosensor was applied to characterize the antimicrobial activities of various antibiotics and the antibiotic susceptibility of *Escherichia coli* W1485. The label-free biosensor allows for both end-point and real-time measurements of antibiotic effects on the bacterial cell surface of LPS and could be a promising tool for the therapeutic management of antibacterial drugs and antibiotic research and development.

Nagaraj et al. (2010) created a miniature label-free electronic biosensor for biomarker detection. It was an ultrasensitive diagnostic platform, called NanoMonitor, to enable the rapid, highly sensitive, and selective label-free analysis of glycans. The NanoMonitor biosensor was made of a silicon chip with an array of gold electrodes that form multiple sensor sites, and it works on the principle of electrochemical impedance spectroscopy. The *Sambucus nigra* agglutinin and *Maackia amurensis* agglutinin (MAA) were conjugated to the surface of the electrode. The binding of specific glycans from a test sample to lectins resulted in an impedance change. NanoMonitor was used to study the glycosylation of fetuin and extracts from a human pancreatic cancer line serum protein. The authors demonstrated that the developed biosensor could rapidly identify the glycoforms of fetuin and differences in the glycosylation of human

pancreatic cancerous and normal cells requiring only 10 μL of a sample. The obtained results correlated well with those of the lectin-based microplate analysis.

Developments in the field of photometric detection of microorganisms using lectins immobilized on the surface of anisotropic silver nanoparticles (AgNPs) or their hybrids with CdSe QDs are presented in a series of studies carried out by Gasparyan and his colleagues. In Gasparyan and Bazukyan's (2013) study, *E. coli*, *B. subtilis*, and *S. aureus* cells were detected with the use of AgNPs sensitized with potato lectin by changes in the optical spectra of nanoparticles upon their interaction with bacteria. The lowest detection limit was observed for *S. aureus* (1.5×10^4 cells/mL). In Mikaelyan et al. (2017), WGA and LCA were immobilized on the surface of AgNPs. Interaction of these lectins with *S. aureus* and *E. coli* resulted in the stabilization of AgNPs and prevented their aggregation upon sodium chloride addition. The stabilization was characterized by a concentration-dependent manner. As a result, the authors achieved the lowest detectable concentrations of *S. aureus* and *E. coli* of 10^3 cells/mL. A hybrid system of QDs–AgNPs sensitized by the lectin from hen eggshell as a recognition ligand was applied in the study of Hovhannisyan et al. (2017) for the detection of *S. aureus*. Fluorescent changes of QDs as a result of resonance energy transfer between AgNPs in their interaction with bacteria were detected. Application of the hybrid provided high-sensitivity analysis and allowed for *S. aureus*' detection limit of 6×10^3 cells/mL.

Lectin biosensors are nowadays hardly commercialized technology; their use is limited to laboratory developments. Compared with lectin-based microarrays, label-free lectin biosensors are characterized by a higher sensitivity and can be successfully used for quantitative analysis. However, a high-performance, multiplexed analysis can be implemented using lectin

microarrays. Therefore, lectin-based microarrays are advisable mainly for the characterization of the carbohydrate composition of unknown glycoproteins, while lectin biosensors should be used to detect definite compounds.

4.7. Lectin-based microplate and lateral flow assay

Among analytical lectin-based approaches, the microplate enzyme-linked lectinosorbent assay (ELLSA) should be mentioned (see different ELLSA formats in Figure 6). As a direct adaptation of the enzyme-linked immunosorbent assay (ELISA), ELLSA was originally based on lectin binding to specific antibodies or the inhibition of this reaction with the corresponding saccharides and used to study lectin–carbohydrate interactions (Duk et al., 1992). However, when characterizing carbohydrates' interactions with several lectins, there was a need for antibodies specific to each of them, which was an important drawback of this method. Therefore, Goodarzi and Turner (1997) proposed an alternative approach using lectins labeled by digoxigenin (DIG) and detected using DIG-specific antibodies. This method remains popular to this day.

Another convenient way of introducing the label is based on the biotin–avidin interaction characterized by high affinity. Here, biotinylated lectins interact with glycoproteins or other high-molecular glycoconjugates noncovalently adsorbed in the wells of a microtiter plate. The formation of complexes is detected using (strept)avidin–enzyme conjugates (Duk et al., 1994). ELLSA, with the "biotin–avidin" system, was proposed to study the fine specificity of bacterial lectins in the studies of Singh et al. (2006) and Wu et al. (2003).

Microplate analysis of cells and carbohydrate structures exposed on cell walls based on carbohydrate–lectin interactions or combining these interactions with immune reactions is of great interest. Several works have been devoted to the determination of carbohydrates in biofilms produced by various microorganisms. Thomas et al. (1997) carried out a qualitative determination of the *N*-acetylglucosamine residues of *Staphylococcal epidermis* responsible for intercellular adhesion. For this purpose, biofilms produced by *S. epidermis* were immobilized in microplate wells, and colorimetric detection was performed using a phosphatase conjugate of WGA specific to *N*-acetylglucosamine. Leriche et al. (2000) developed ELLSA to detect and characterize extracellular polysaccharides produced by the biofilms of 10 bacteria strains. HRP-labeled Con A was used for the colorimetric detection of *D*-glucose and *D*-mannose, which are frequently presented in biofilms. Peroxidase conjugates of WGA were used to detect *N*-acetyl-*D*-glucosamine and *N*-acetylneuraminic acid. The developed analysis allowed not only for the detection of *Stenotrophomonas maltophilia* and *Staphylococcus sciuri* but also for the quantification of the dynamics of Con A- and WGA-specific exopolysaccharide production. Strathmann et al. (2002) used ELLSA to characterize carbohydrate-containing extracellular

polymeric compounds in *Pseudomonas aeruginosa* biofilms. As in the previous work, the carbohydrate components of biofilms were colorimetrically detected with Con A–HRP conjugates. Graham et al. (1984) developed ELLSA for the detection of *Bacillus anthtacis* and its differentiation from the closely related *B. cereus* and *B. cereus* var. *mycoides*. SBA and HPA were conjugated to peroxidase. The formation of target complexes with immobilized bacterial cells was detected colorimetrically. The detection limit was 2.6×10^4 cells.

Hendrickson et al. (2017) performed ELLSA in a "sandwich" format to detect *Escherichia coli* and *Staphylococcus aureus* bacterial cells. The specificity of several plant lectins to these pathogens was studied; lectins characterized by high-affinity binding to cells were selected. As a result, ELLSA was carried out using WGA and RCA. The detection limits of *E. coli* and *S. aureus* were 4×10^6 and 4×10^5 cells/ml, respectively. A comparison of two formats of *E. coli* assay—ELISA and ELLSA—revealed no reliable differences in detection limit values.

Ching et al. (1989) developed ELLSA using PNA specific to the oncomarker–glycoprotein containing a carcinoembryonic antigen of galactose 1-3 *N*-acetylgalactosamine. Peroxidase-labeled PNA was incubated with sera from pancreatic cancer patients immobilized in microplate wells. Detection of the enzymatic activity of the formed immune complexes was carried out colorimetrically. The developed analysis allowed for the determination of PNA-reactive glycoprotein in the serum of patients with high reproducibility, sensitivity (77%), and specificity (92%) and showed a high correlation with radioimmunoassay.

A combination of immune and lectin–carbohydrate interactions to determine carbohydrates and glycoconjugates was applied in several studies. Such analytical systems were developed to detect various pathologies. Abushoufa et al. (2000) developed a "sandwich"

ELLSA to determine glycogen forms of human chorionic gonadotropin (hCG) in blood serum for the prenatal diagnosis of Down's syndrome. In this assay, the analyzed samples and WGA–HRP conjugates specific to sialic acid in hCG glycoform were serially incubated with anti-hCG antibodies immobilized in microplate wells. The working range of the analysis was 100 – >500 000 IU/I. McDowell et al. (2001) developed a "sandwich" ELLSA to detect low-density lipoproteins (LDL) in the blood of patients with coronary artery disease. In this assay, LDL was determined by deglycosylated anti-LDL antibodies immobilized in microplate wells, biotinylated RCA, and streptavidin-alkaline. The working range of the assay was 0.05–3 μg/ml.

Parker et al. (1992) detected mucus glycoproteins in blood serum using a "sandwich" ELLSA to diagnose pancreatic cancer. Unlike the above formats, WGA was absorbed in the solid phase. That analyte reacted with WGA was detected using specific antibodies and anti-species enzyme-labeled antibodies. The sensitivity and specificity of the developed analysis was 78% and 76%, respectively. An interesting approach for the detection of α-2.6-sialated transferrin (TF) glycoforms was developed by Hoshi et al. (2013). The proposed analysis format was based on the inhibition of the interaction of TF with anti-TF specific antibodies labeled with peroxidase by SSA. Bronzoni et al. (2005) applied a "sandwich" ELLSA to detect the bronchitis virus and its specific antibodies with Con A immobilization in microtiter plate wells. The detection limit of the antigen was $10^{5.1}$ EID_{50}/ml.

Only a few studies have described the use of nanoparticles as markers for recording the carbohydrate–lectin complexation. Sun et al. (2014) proposed an interesting approach for detecting alpha-fetoprotein as the oncomarker of hepatocellular carcinoma that involved

measuring the spectral characteristics of LCA-immobilized 15-20-nm gold nanoparticles change upon binding with alpha-fetoprotein. The detection limit was 100 ng/ml.

Only one investigation devoted to the development of lateral flow analysis (LFA) of glycans can be found in the literature thus far (Damborský et al., 2016). The authors for the first time demonstrated the possibility of LFA development using lectins as biorecognition elements instead of antibodies or nucleic acids. They developed a lectin-based LFA strip to determine the glycosylation status of free PSAg as a more specific marker of disease progress than only PSAg level. SNA and MAL were used to specifically detect α-2.6 and α-2.3 sialic acids. This novel approach can be applied to a wide range of biomarkers as an easily performed test that allows result attainment within 10 minutes.

5. Conclusion

The presented data demonstrate the great potential of lectins as components of analytical systems of various formats. Lectin-based analytical systems can either be independent analytical tools or act as complements to instrumental research. The wide distribution of lectins allows for their simple isolation and purification and makes them highly useful available components of diagnostic test systems.

List of abbreviations

AAL – *Aleuria aurantia* lectin

AFP – alpha-fetoprotein

AgNPs – silver nanoparticles

BLF – bovine lactoferrin

Con A – concanavalin A

CRD – carbohydrate recognition domains

CT – cholera toxin

DIG – digoxigenin

ECoRL – *Erythrina corallodendron* lectin

EEL – *Euonymus europaeus* lectin

EIS – electrochemical impedance spectroscopy

ELISA – enzyme-linked immunosorbent assay

ELLSA – enzyme-linked lectinosorbent assay

ENO – enolase

ESCs – embryonic stem cells

FRET – fluorescence resonance energy transfer

Fuc – fucose

Gal – galactose

GalNAc – *N*-acetylgalactosamine

Glc – glucose

GlcNAc – *N*-acetylglucosamine

GNA – *Galanthus nivalis* agglutinin

GSL – *Griffonia simplicifolia* lectin

hCG – human chorionic gonadotropin

HPA – *Helix pomatia* agglutinin

HRP – horseradish peroxidase

Hsp – heat shock protein

iPSCs – induced pluripotent stem cells

LCA – *Lens culinaris* agglutinin

LDL – low-density lipoproteins

LFA – lateral flow analysis

LOL – *Lathyrus ochrus* lectin

LPS – lipopolysaccharide

MAL – *Maackia amurensis* lectin

Man – mannose

MOA – *Marasmius oreades* agglutinin

MV – microvesicles

PHA – *Phaseolus vulgaris* agglutinin

PhHA – phytohemagglutinin

PNA – peanut agglutinin

PSAg – prostate-specific antigen

PSA – *Pisum sativum* agglutinin

QCM – quartz crystal microbalance

QDs – quantum dots

RCA – *Ricinus communis* agglutinin

RSL – *Ralstonia solanacearum* lectin

SBA – soybean agglutinin

SCs – somatic cells

Sia – sialic acid

SNA – *Sambucus nigra* agglutinin

SPR – surface plasmon resonance

SSA – *Sambucus sieboldiana* agglutinin

TF – transferring

UEA – *Ulex europaeus* agglutinin

WGA – wheat germ agglutinin

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Table 1. Plant and fungi lectin families according to Lannoo and Van Damme (2014), Van Damme (2014), and the Lectin Frontier Database (http://acgg.asia/lfdb2/).

Plant lectin family		Examples of lectins	Type of protein molecule fold
1	*Agaricus bisporus* homolog	*Agaricus bisporus* agglutinin, *Xerocomus chrysenteron* agglutinin, *Pholiota squarrosa* lectin	β-sandwich
2	Amaranthin domain	*Amaranthus caudatus agglutinin, Amaranthus spinosus agglutinin*	β-trefoil
3	Homolog of class V chitinases	*Robinia pseudoacacia agglutinin*	TIM barrel
4	Cyanovirin domain	*Ceratopteris richardii* lectin	Triple-stranded β-sheet and a β-hairpin
5	*Euonymus europaeus* lectin domain	*Euonymus europaeus* agglutinin	Unknown structure
6	*Galanthus nivalis* agglutinin domain	*Galanthus nivalis* agglutinin, *Hippeastrum*	β-barrel

		hybrid agglutinin, *Narcissus pseudonarcissus* agglutinin	
7	Hevein domain	Datura stramonium agglutinin, *Lycipersicon esculentum* lectin, *Phytolacca americana* agglutinin, *Solanum teberosum* agglutinin	Hevein
8	Jacalin domain	Jacalin, artocarpin, *Maclura pomifera* agglutinin, *Helianthus tuberosus* agglutinin	β-prism
9	Legume domain	Con A, DBA, LCA, PNA, SBA, *Wisteria floribunda* agglutinin, *Vicia villosa* agglutinin	β-sandwich
10	Lysin motif (Lys M) domain	*Pteris ryukyuensis* lectin, *Medicago truncatula* lectin	β-α-α-β-structure
11	Nictaba-like domain	*Nicotiana tabacum* agglutinin, *Oryza sativa lectin*,	Unknown structure
12	Ricin-B domain	RCA, MOA, SNA, SSA, *Trichosanthes japonica* agglutinin	β-trefoil

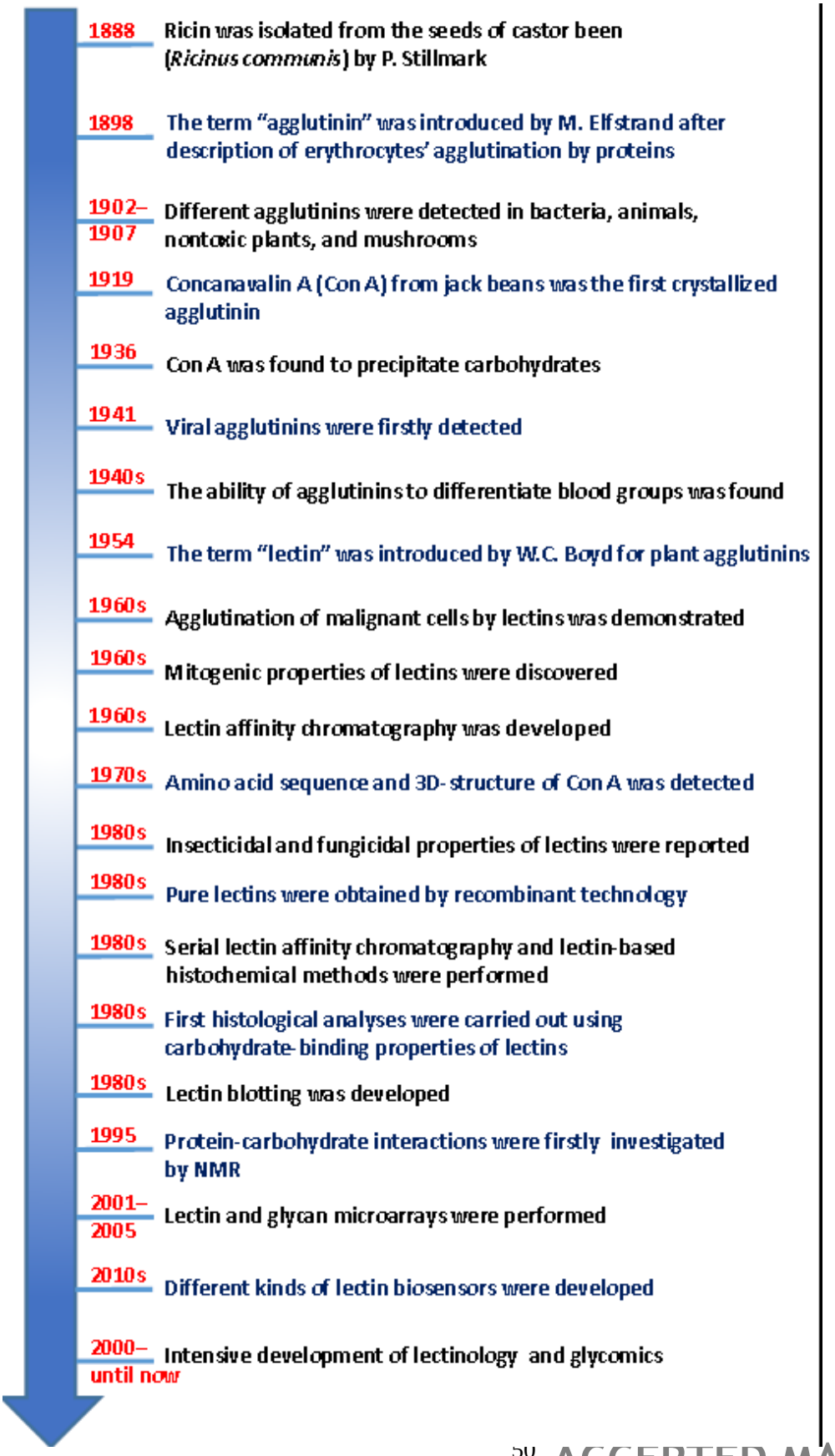

Figure 1. Main milestones of the history of lectin research.

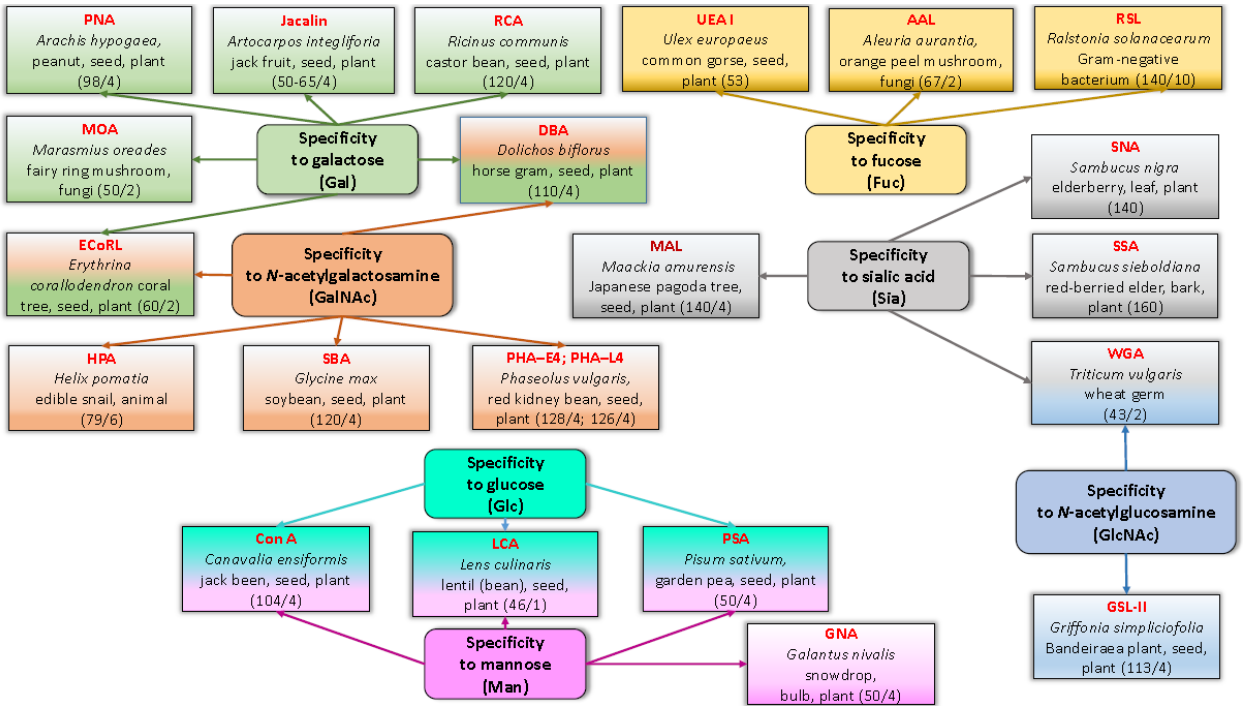

Figure 2. Specificity of some lectins according to the Lectin Frontier Database (http://acgg.asia/lfdb2/). Each square contains the abbreviation of a lectin and the name of its origin. The molecular weight (kDa) and the number of oligomers in the lectin molecule are indicated in parentheses.

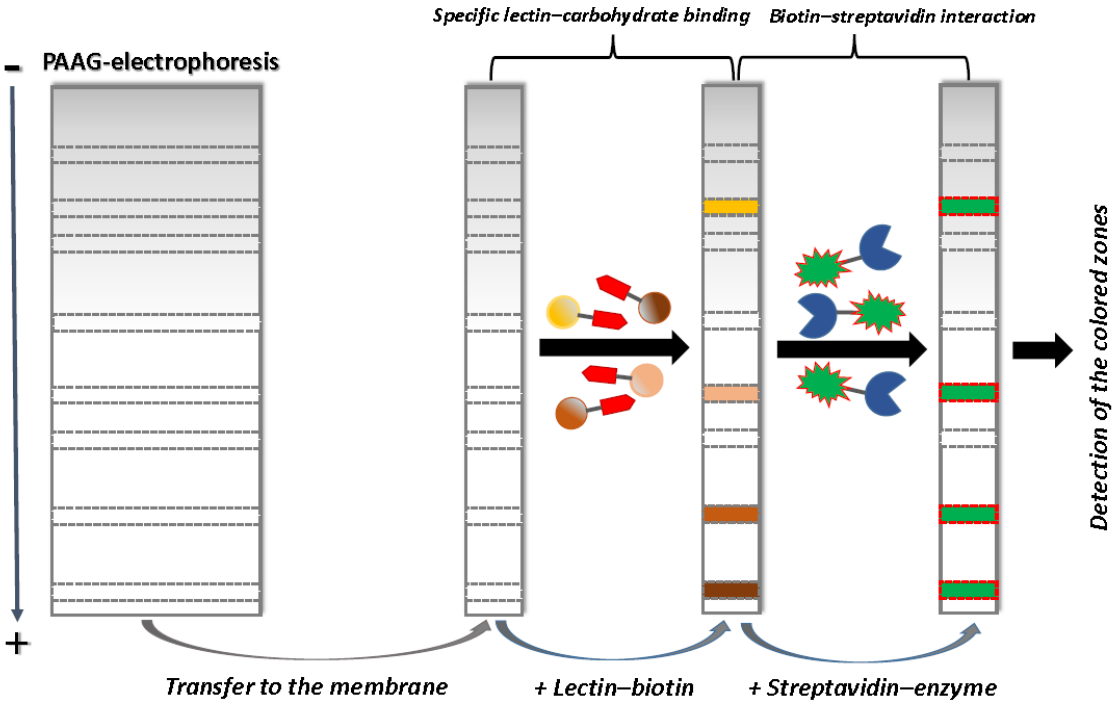

Figure 3. Scheme of lectin blotting.

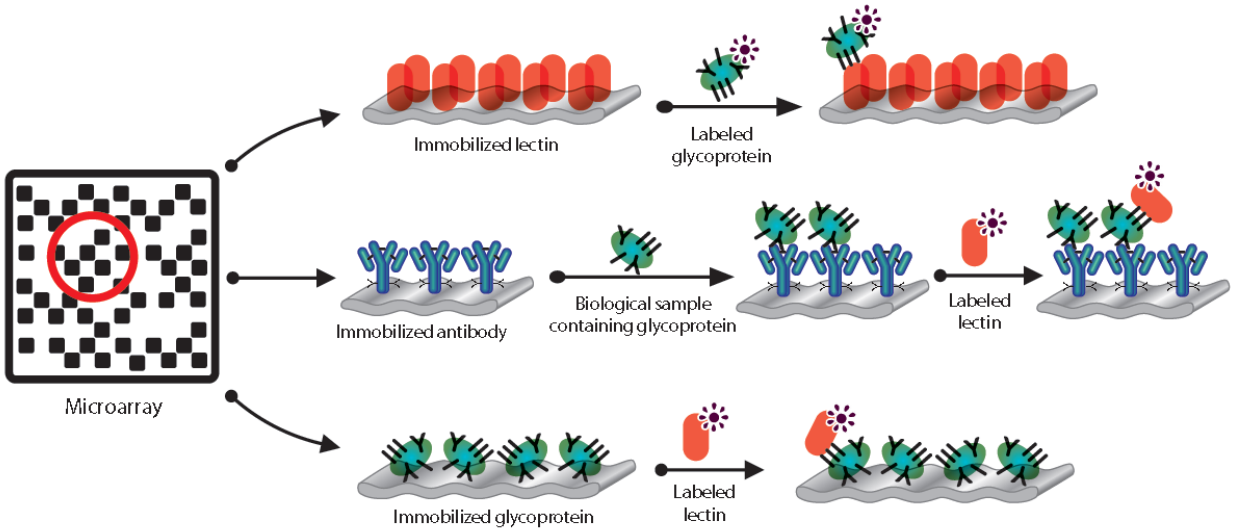

Figure 4. Lectin microarray in different modes.

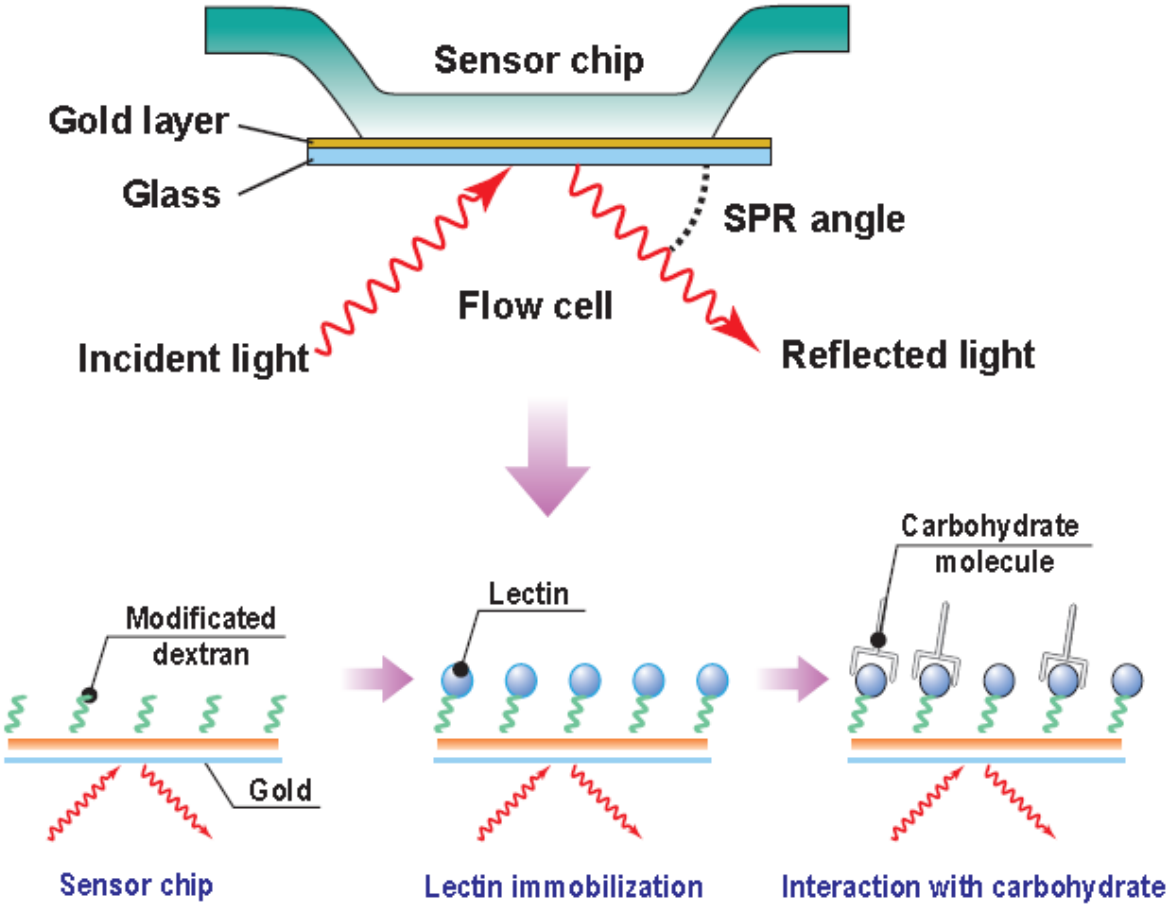

Figure 5. Detection of lectin–carbohydrate interaction by SPR biosensor.

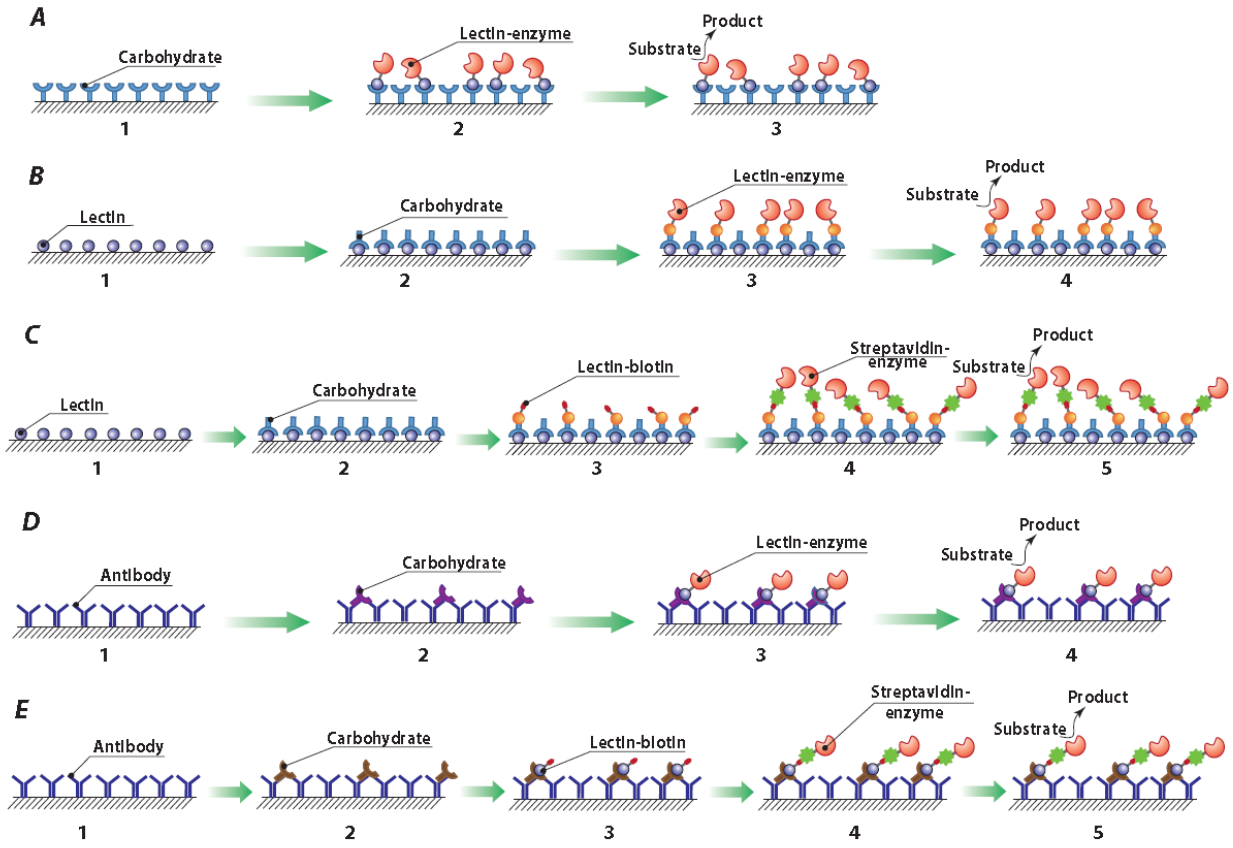

Figure 6. Different formats of ELLSA: A. a format with immobilized carbohydrates; B. a "sandwich" format with immobilized lectin and lectin-enzyme conjugate; C. a "sandwich" format with immobilized lectin, lectin-biotin, and streptavidin-enzyme conjugate; D. a "sandwich" format with immobilized anti-carbohydrate antibodies and lectin-enzyme conjugate; E. a "sandwich" format with immobilized anti-carbohydrate antibodies, lectin-biotin, and streptavidin-enzyme conjugate.