Review Article

Phythemagglutinins

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Introduction

Plant constituents of low molecular weight often interact strongly with biological systems. Many of them are widely used in therapy. High molecular weight constituents with strong biological effects, however, have attracted the attention of investigators only during recent decades. One group of these plant compounds, the phythemagglutinins, is the subject of the present article.

Nearly a century ago, H. STILLMARK from the University of Dorpat (Estonia) described the agglutinating ability towards red blood cells of some Euphorbiaceae seed extracts. The plant which later gained most attention is the castor bean (*Ricinus communis*). It is extremely toxic to man and animals. At that time, STILLMARK was not able to purify the substances responsible for agglutination, but he already supposed them to be proteins [1].

During the following decades, phythemagglutinins were detected in many other seed extracts, also in nontoxic ones. Most of these proteins were found in the Leguminosae family, e.g. concanavalin A (conA) from Jack beans (*Canavalia ensiformis*), favin from the broad bean (*Vicia faba*) and phasin (or PHA, "phythemagglutinin" proper) from the kidney bean (*Phaseolus vulgaris*). ConA was one of the first proteins crystallized (Summer 1919).

Agglutination by most of these proteins could be inhibited by simple sugars, oligosaccharides, glycosides or glycoproteins. Moreover, interaction, i.e. precipitation, was also demonstrated with glycoproteins and polysaccharides.

Concanavalin A has now become the most prominent phythemagglutinin. It has found many applications in cytology and immunology, even a monograph has been dedicated to this protein [2].

STILLMARK already recognized that ricin, the agglutinin from the castor beans, agglutinates red blood bodies from various animals with different intensities. Though this also applies to other phythemagglutinins, these proteins generally proved to be unspecific towards human blood groups.

Since the fifties, however, several phythemagglutinins were detected which specifically react with either human group A or group O erythrocytes, whereas blood group B specific agglutinins are still very scarce. Moreover, agglutinins directed towards bacteria, yeasts and other cells were described. The development in this field is covered in several reviews [1, 3–7].

The occurrence of blood group specific phythemagglutinins led to the proposal put forward by BOYD and SHAPLEIGH [8] to call these proteins "lectins" (from Latin: legere = to choose, to pick out). Boyd wanted to express his expectation that the blood group specificity might be found more generally. Though this hope was not fulfilled, as most phythemagglutinins detected earlier and later on are unspecific, the name *lectin* has persisted and is now in use as a synonym for agglutinins, not only from plant sources but also from microorganisms, fungi, animals and humans. The question about the definition of a lectin is now under discussion [9, 10], but it is generally accepted that a lectin agglutinates some type of cell, either native or enzymatically modified, or at least interacts with them, and that this interaction can be inhibited by sugars. Lectins do not exhibit enzymatic activities, i.e. they do not alter the chemical nature of the sugar residues to which they bind.

Of course, any classification of this kind will be artificial because it necessarily has to be based upon *in vitro* observations. On the one hand, there are monovalent sugar binding proteins which are closely related to lectins, as e.g. the monovalent, non-agglutinating Ricinus toxin to the divalent Ricinus agglutinin [11]. On the other hand, slowly acting oligomeric forms of glycosidases occasionally may first bind to cells thereby agglutinating them, and only after some hours' standing hydrolyze the sugar determinant from the cell surface thus releasing the cells again [12].

This disagreement in defining lectins only reflects the uncertainty about the biological role of these proteins. There are, however, several strong arguments in favour of a biological role (see below).

Occurrence

Most lectins are detected in and isolated from seeds. In fact, most plant lectins are localized in the mature seed. The occurrence in other parts of the plant has been investigated only sparingly and often the results are contradictory. On the whole, it seems that little if any lectin is found outside the seeds [13–15]. Nevertheless, very little lectin or material with immunological crossreactivity to lectins was found on root hair surfaces. This may be of importance in view of the supposed biological role of the Leguminosae lectins, i.e. interaction with nodulating bacteria [16, 17]. From our experience [18], most of the lectin in a germinating Leguminous plant (pea) is found in the cotyledons. During germination, the cotyledons lose their lectin. The other parts of the growing plant contain little, not equivalent amounts. Only after flowering does the plant synthesize or accumulate lectin in the young fruit. Within the cotyledons, the bulk of lectin is found in the soluble portion of the cells [18–20]. Other authors, however, report that lectins are associated with the protein bodies, i.e. they form part of the storage proteins [21].

Most lectins have been isolated from the Leguminosae. In Table I, some frequently used lectins are shown. Also other plant families have contributed to this protein group (Table II). Accounts on this subject have been given by several authors [1, 3–6], most recently by GOLDSTEIN and HAYES [7].

Interaction with sugars, chemical structure

Lectins by definition interact with sugars. Though in most instances a simple sugar, usually a monosaccharide, is known which interacts with a particular lectin, and which consequently inhibits agglutination of cells and even dissociates agglutinated cells, this specificity towards sugars mostly is not paralleled by a cell specificity. This only means that lectins are directed towards determinants which are common to most cell surfaces. Only a few lectins interact with the ABO determinants thus justifying their name (Table III). As expected, agglutination by these lectins is specifically inhibited by the terminal sugar of the respective blood group substance. It is interesting to note that three plants bearing a blood group specific lectin (*Vicia cracca*, *Bandeiraea simplicifolia* and *Ulex europaeus*) additionally contain unspecific lectins which differ from the specific ones in their sugar binding specificity. In the case of *Vicia cracca* which was investigated by our group more thoroughly [22–25], the unspecific, mannose/glucose binding lectin appears in all specimens collected at various places in nearly equal concentration, whereas the amount of the specific lectin is very different from one sample to another.

By their chemical nature, lectins are proteins, most of them glycoproteins, containing 2–50 % carbohydrate. Only concanavalin A and the wheat germ agglutinin (WGA) are devoid of sugar. Evidently, a glycosyl moiety is not necessary for a lectin to interact with sugars. Only few lectins have been analyzed for their carbohydrate composition in detail. The Leguminosae lectin investigated in this respect do not differ significantly from other plant or animal glycopro-

Table I

Some lectin containing Leguminosae

Plant species	lectin (most frequently used name)	sugar specificity ¹⁾
<i>Canavalia ensiformis</i> (Jack bean)	concanavalin A (ConA)	α -Man, α -Glc
<i>Vicia faba</i> , <i>fava</i> (broad bean)	favin	α -Man, α -Glc
<i>Arachis hypogaea</i> (peanut)	peanut agglutinin (PNA)	α , β -Gal
<i>Glycine max</i> (soybean)	soybean agglutinin (SBA)	α , β -GalNAc
<i>Lotus tetragonolobus</i> (asparagus pea)	Lotus lectin	α -Fuc
<i>Phaseolus vulgaris</i>	(Phaseolus) phythem- agglutinin (PHA)	complex oligo- saccharide

¹⁾ Abbreviations: Man: D-mannose; Glc: D-glucose; Gal: D-galactose; GalNAc: N-acetyl-D-galactosamine; Fuc: L-fucose; GlcNAc: N-acetyl-D-glucosamine

Table II

Distribution of lectins in the plant kingdom

family	species	sugar specificity ¹⁾
fungi		
Agaricales	<i>Agaricus bisporus</i>	complex
flowering plants		
<u>Dicotyledoneae</u>		
Moraceae	<i>Maclura pomifera</i>	GalNAc, Gal
Loranthaceae	<i>Viscum album</i>	α , β -Gal
Phytolaccaceae	<i>Phytolacca americana</i>	unknown
Cruciferae	<i>Iberis amara</i>	GalNAc, Gal
Leguminosae	see Table I	
Euphorbiaceae	<i>Ricinus communis</i>	β , α -Gal
Celastraceae	<i>Euonymus europaeus</i>	complex
Labiatae	<i>Salvia horminum</i>	unknown
Solanaceae	<i>Solanum tuberosum</i>	GlcNAc ^{β, 1,4} GlcNAc and higher oligomers
<u>Mono-</u> <u>cotyledoneae</u>		
	<i>Triticum vulgare</i>	GlcNAc ^{β, 1,4} GlcNAc and higher oligomers
Gramineae		

¹⁾ For abbreviations see Table I

teins. They contain high mannose type oligosaccharide side chains anchored to an asparagine residue of the protein by a N-acetylglucosamine bridge [26–30]. Partial removal of the sugar residues seems not to impair the biological activity [30]. The glycosyl moiety may contribute to the microheterogeneity which is often found in lectins. The potato lectin is quite uncommon in its carbohydrate composition [31, 32]. It contains about 50 % carbohydrate, predominantly L-arabinose bound to 4-hydroxyproline residues. Besides, little serine-bound galactose occurs.

Being glycoproteins is nearly the only common property of the bulk of lectins. All other data such as molecular size and architecture as well as amino acid composition are very different (Table IV). The lectins which have been studied most intensively are those from the Leguminosae. On the one hand, there are lectins with various sugar specificities which are made up of four nearly or totally identical peptide chains. ConA and PHA, the kidney bean lectin, belong to this group. Subunit molecular weights range from about 25 000 to 35 000, resulting in total molecular weights of 100 000–140 000. On the other hand, there is a group of lectins which specifically bind

mannose, glucose and their α -glycosides. These lectins are composed of two pairs of different chains, leading to a $\alpha_2\beta_2$ structure, with molecular weights of 5000–7000 (α -chain) and 14 000–20 000 (β -chain), hence their total molecular weight is 40 000–50 000. The latter group is only found in the Viciaeae tribe. These proteins are called two chain lectins, in contrast to the one chain lectins mentioned first [33], (Figure 1). Some of these lectins are compiled in Table V.

Vicia cracca is a remarkable plant since it contains lectins of either type [22–24]. The one chain lectin is specific towards human blood group A and binds to N-acetylgalactosamine, the two chain lectin is unspecific in the AB0 system and binds to mannose/glucose. Remarkably, both lectins from *Vicia cracca* interact with each other as evidenced by affinity chromatography and laser nephelometry [25]. This *per se* is not surprising since both lectins are glycoproteins. It has, however, to be kept in mind that they are constituents of the same plant and occur in the same cell compartment, namely in the soluble portion [20].

As is generally known, relationships between proteins are reflected in their amino acid sequences. Some proteins are conservative, i.e. they tolerate only a few replacements during evolution and thus are tightly related even in distant species. On the other hand, many proteins have evolved very quickly and have become quite different even in related species. The lectins from the Leguminosae have to be regarded as conservative proteins. The α -chains of several two chain lectins are nearly identical [24, 34]. Even the homologous portion of a one chain lectin (ConA) appears to be quite similar. The degree of relationship can be condensed into one figure according to MAC-LACHLAN [35] and SCHILTZ et al. [36]. Depending upon the degree of relationship, the McLACHLAN indices range from 0 to 1 (Table VI).

Though only few β -chains of one chain lectins have

Table III

Blood group (AB0) specific lectins

Blood group A, binding to α -GalNAc:	<i>Vicia cracca</i> , <i>Phaseolus lunatus limensis</i> , <i>Dolichos biflorus</i>
Blood group B, binding to α -Gal:	<i>Bandeiraea simplicifolia</i> (one of the isolectins)
Blood group O, binding to α -L-Fuc:	<i>Lotus tetragonolobus</i> , <i>Ulex europaeus</i>

Table IV

Properties of lectins from different plants

family	species	molecular weight and architecture	remarks	% carbohydrate
Leguminosae	<i>Phaseolus vulgaris</i>	120 000 (4 × 30 000)	no cysteine and methionine	4–10
	<i>Canavalia ensiformis</i>	104 000 (4 × 26 000)	no cysteine and methionine	0.0
	<i>Pisum sativum</i>	50 000 (2 × 7 000 + 2 × 17 000)	no cysteine and methionine	0.2–1
Euphorbiaceae	<i>Ricinus communis</i>	120 000 (2 × 31 000 + 2 × 34 000)	subunits held together by disulphide bonds	12
Solanaceae	<i>Solanum tuberosum</i>	100 000 (2 × 50 000)	rich in hydroxyproline and cysteine, contains ornithine	50
Gramineae	<i>Triticum vulgare</i>	36 000 (2 × 18 000)	rich in cysteine and glycine	0.0

Fig. 1

Table V

One chain and two chain lectins from Leguminosae

species	tribe	molecular weight and architecture
<i>Canavalia ensiformis</i>	Phaseoleae	104 000 (4×26 000)
<i>Glycine max</i>	"	120 000 (4×30 000)
<i>Phaseolus vulgaris</i>	"	120 000 (4×30 000)
<i>Vicia cracca</i>	Vicieae	125 000 (4×33 000)
<i>Vicia cracca</i>	"	44 000 (2× 5 700+2×17 500)
<i>Vicia sativa</i>	"	40 000 (2×6 000+2×14 000)
<i>Vicia faba</i>	"	51 000 (2×5 600+2×20 000)
<i>Pisum sativum</i>	"	50 000 (2×7 000+2×17 000)
<i>Lens culinaris</i>	"	46 500 (2×5 700+2×17 500)

Table VI

Comparison of the amino acid sequences by the Mc Lachlan index (data from [24] and [34]).

	Vc	Ps	Vs	Vf	Lc	ConA
<i>Vicia cracca</i> (Man)	1.000					
<i>Pisum sativum</i>	0.976	1.000				
<i>Vicia sativa</i>	0.937	0.937	1.000			
<i>Vicia faba</i>	0.901	0.901	0.887	1.000		
<i>Lens culinaris</i>	0.887	0.887	0.882	0.848	1.000	
<i>Canavalia ensi-</i> <i>formis</i> (ConA)	0.679	0.720	0.717	0.698	0.695	1.000

Fig. 2. Circular permutation of the ConA and favin sequences (according to [37]).

been sequenced up to now, a high degree of homology has also been observed in this case [37, 38]. Remarkably, β -chain of the mannose/glucose binding lectin and the N-acetylgalactosamine binding lectin from *Vicia cracca* differ much more (McLachlan index 0.779) than β -chains of different one chain lectins [23].

Relationships between proteins can also be detected by immunoprecipitation. This method primarily reveals similarities in the secondary and tertiary structures which of course reflect homologous amino acid sequences. We raised antibodies against several lectins and studied crossreactions in double diffusion [39]. It turned out that all two chain lectins crossreact with each other, in part by forming a spur, whereas the one chain lectins neither respond to antibodies directed against two chain lectins nor against other one chain lectins. It has to be emphasized that even the lectins from *Vicia cracca* do not crossreact.

From this and from the amino acid sequences it has to be concluded that lectins from different plants but with the same sugar specificity may be more closely related to each other than lectins from the same plant but different in their sugar specificity.

Though not highly homologous, one chain and two chain lectins display an exciting relationship [37, 38]. If the one chain lectin (ConA) is presented as a circle, and the two chains lectin (favin) aligned to the homologous residues in a parallel circle, the gaps in either circle are overlapped by the other one (Fig. 2). At present, only speculations are possible as to what kind of DNA rearrangement has led to this circular permutation.

In spite of the fact that much is known about occurrence, localization and chemical structure of phythemagglutinins, their biological function is largely unknown. This contrasts with the lectins from animals and microorganisms which though often poorly characterized by their chemical structure are much better understood in terms of their function. It is, however, without doubt that plant lectins also play a rôle in Nature. The Leguminosae lectins are conservative in their amino acid sequence and sugar binding site, they occur in considerable amounts and, within the plant, they are associated with proteins which bind specifically to them. These proteins, called lectin binders by us [40–42] and lectin receptors by other authors [43] are found in the same order of magnitude as the lectins. At present, however, the funtion of phythemagglutinins remains an open question.

Application

Lectins have found a continuously growing interest. Several international conference series have been devoted to this topic, in particular to diagnostic and clinical application.

Most serum proteins are glycoproteins, and their pattern changes depending on the physiological state of the organism. Therefore immobilized lectins have frequently been used in order to "fingerprint" serum proteins with respect to kind and amount of glycosylation. Glycoproteins are precipitated by lectins under certain circumstances. Therefore their amount can easily be determined by nephelometry. KÖTTGEN et al. [44] and WARREN et al. [45] describe this method and its application to clinical problems. Alternatively, electrophoretical techniques may be used. Glycoproteins can be subjected to countercurrent electrophoresis in agarose gels or cellulose acetate membranes either against specific antibodies or against lectins [45, 46] if antibodies are not available. BØG-HANSEN [47] and BØG-HANSEN and TAKEO [48] introduced affinity electrophoresis which is a two dimensional technique. These authors make use of the phenomenon that a glycoprotein moving in an electrical field is retarded by an immobile or slowly moving lectin depending upon its glycosylation degree. In a second run, the separated glycoproteins are subjected to electrophoresis perpendicularly to the first direction into a gel which contains antibody against the original glycoprotein mixture. The precipitation bands forming provide information about the composition of the original mixture. By this means, correlations could be revealed between glycosylation of proteins from serum or other body fluids and the state of health of the donor [49].

Agglutination of red blood bodies by specific lectins may be used for blood group typing. For this purpose, most clinicians prefer human antibodies, but this is prohibitive in studying blood group distribution in large population samples because of the costs. Lectins, in most cases crude seed extracts, are much less expensive than antibodies and totally meet these demands.

Lectins have been used for characterizing the carbohydrate composition of cell surfaces. Thus, making use of fluorescent lectin derivatives, BRABEC et al. [50] showed that the epidermis layers of newborn rats are equipped with different carbohydrate determinants. Basal and lower spinous cells are labelled by a lectin from *Bandeiraea simplicifolia* which specifically binds to α -galactosyl residues. Spinous cells apparently are covered with α -L-fucosyl residues since they bind to *Ulex europaeus* (gorse, furze) lectin, whereas a second, N-acetylglucosamine binding lectin from *Bandeiraea simplicifolia* is found at the stratum corneum cells.

Much attention has been directed to the question as to whether malignant cells can be differentiated from normal ones by use of lectins. It is generally accepted that malignant cells have an altered surface because they are not subject to contact inhibition. It has been known for many years that some lectins preferentially agglutinate malignant cells, i.e. these cells

are agglutinated at a 3–100 fold lower lectin concentration than normal ones [4]. The wheat germ lectin (WGA), ConA and the lectins from soybeans and kidney beans were used in most studies. These lectins are also active against cells which just undergo mitosis, i.e. a malignant cell can be regarded as a dividing cell which does not return to the resting state.

From the bulk of experiments, only a few will be discussed here. As REISNER et al. [51] found out, the peanut lectin does not react with normal human peripheral lymphocytes. It agglutinates, however, the immature thymocytes. The same holds true for peripheral lymphocytes in acute leukemia, whereas chronic leukemia lymphocytes do not respond to the peanut lectin. From these results, it may be concluded that the peanut lectin receptor is exposed in the immature and in the acute leukemia cells, whereas it is masked in mature lymphocytes. It is quite evident that the ability to differentiate between these two lymphocyte types may be of great diagnostic value. Even a therapy may be developed from these observations: If it were possible to covalently couple a cytostatic agent to a lectin specific for transformed cells, and if the drug would enter the cell only after having been bound to it *via* the lectin, the drug action could be restricted to the target cell. This would greatly reduce the dose which is necessary to achieve a therapeutic effect, thereby eliminating unwanted side effects. First attempts in this direction with lectin-cytotoxin conjugates have been quite promising [52–54].

Unfortunately, not all types of malignant cells do respond to the lectins mentioned. Moreover, the effect is a quantitative rather than a qualitative one. Therefore, expectations to develop a simple and general diagnostic method have not been realized, not to speak of a general therapeutic application.

Lectins by themselves do not enter cells. There are, however, some remarkable exceptions. The toxic lectin ricin from the castor bean (*Ricinus communis*) consists of two subunits which are held together by a disulphide bridge (Table IV). One subunit, the haptomer, fixes to the cell by surface galactose residues. The other subunit, the effectomer, is now split from the haptomer by reduction somehow taking place on the membrane and in consequence enters the cell. Inside, it acts as a catalyst specifically inactivating one of the elongation factors in protein biosynthesis [55]. This ends up in a catastrophe: The cell will be killed.

At present, many laboratories throughout the world try either to combine the toxic action of the ricin effectomer with a haptomer or an antibody of a peculiar cell specificity or to chemically alter the ricin molecule in order to render it recognizable by the target cell. Thus, UCHIDA et al. [56] coupled the ricin effectomer with a subunit of the *Wistaria floribunda* lectin (N-acetylglucosamine binding). The hybrid toxin/lectin now was directed specifically against N-

acetylglucosamine bearing cell surface structures as evidenced by specific inhibition.

The second approach was taken by YOUNG et al. [57]. They provided ricin with an oligosaccharide chain bearing terminal 6-phosphomannose. This sugar serves as a label for proteins to be taken up by fibroblasts. In fact, the modified ricin entered fibroblasts thereby killing them. In contrast to the native lectin, this process could not be inhibited by galactose, evidencing that the modified lectin does not enter the cell *via* the "normal" ricin receptors. Also by this experiment, it was possible to direct the toxicity to a peculiar cell type.

A quite new application of lectins arose from the observation of NOWELL [58] that lymphocyte cultures after adding the lectin from kidney beans (PHA) undergo enhanced proliferation. Thus this lectin, more precisely one of the isolectins, proved to be a potent mitogen. In the following years many other lectins also turned out to act as mitogens. Besides enhanced proliferation, antibody production by lymphocytes is also stimulated. These effects are mediated by glycoside receptors on the cell surface since the lectin specific sugars act as inhibitors. The mechanism of these effects, i.e. the events which take place between binding of the lectin to the cell surface and stimulation of nucleic acid and protein synthesis, is unknown – as is the case with natural antigens. Evidently, however, lectins need not enter the cell in order to stimulate it. The discovery of NOWELL was a piece of luck for cytology. Though "classical" antigens also are able to stimulate mitosis and antibody production, they only do so in that very small lymphocyte subpopulation which is competent for this particular antigen. Lectins, however, are polyclonal stimulators, therefore their effects are much stronger and now they are frequently used as antigen models. Recently, we found that even fragments of lectins down to small peptides possess a residual but significant stimulatory activity [59].

Thus it should be possible to use mitogenic lectins and their fragments as diagnostic tools either in order to trace hereditary defects in the immune system or to control immunotherapy.

Production of antibodies is governed by a complicated network of different lymphocyte subpopulations, B- and T-cells playing the main part in these events [60]. B-cells excrete antibodies but are informed to do so by T-cells. All mitogenic lectins hitherto described primarily interact with T-cells thereby inducing them to give a signal to the B-cells which in turn respond by proliferation and antibody production. This means that only mixed lymphocyte populations can be affected by lectins.

As mentioned above, we recently discovered that from lectin bearing plants proteins can be isolated which specifically interact with the respective isologous lectin [40–42]. These lectin binders are able to

Table VII

Mitogenic response of lymphocytes to *Vicia faba* or ConA lectin (4 µg/ml) and lectin binder (200 µg/ml) (data from [61], optima response is arbitrarily set to 100).

	mixed cells			B-cells alone (nu/nu)
	without fractionation	after removal of B-cells	in presence of cyclosporin A	
lectin	100	100	4	3
lectin binder	100	10	100	100

stimulate lymphocytes in the same way as is known of the lectins (Table VII). They act, however, at much higher concentrations though the proliferation rates achieved are comparable to those of the lectins. A surprising observation came when we reacted lectin binders with B-cells free from T-cells [61]. Such cells were taken from spleens of nu/nu mice which are genetically defect in lacking a functional thymus. These cells are not stimulated by lectins, but the proliferation rate induced by lectin binders is as high as in normal mixed lymphocytes and occurs at the same mitogen concentration. This not only suggests that B-cells are affected directly but also that T-cells do not participate in this process. This in turn was confirmed by controls:

a specific suppressor of T-cell growth and effector protein production, cyclosporin A, does not influence the mitogenic response of mixed or of pure B-cell cultures to a lectin binder; removal of B-cells from mixed lymphocytes, on the other hand, abolishes this effect.

In investigating complicated systems like the immune system it is of utmost importance to have at hand tools which enable one to resolve the chain of events into single reactions which can be studied separately. In this context, we expect lectin binders to find their place in cytology in the same way as lectins already have done.

Lectins and related proteins from plants, though at first only regarded as a curiosity, have turned out to be valuable tools for studying animal and human immune systems. At present, their application seems to be greatly restricted to analytical and diagnostic purposes. As soon, however, as it is possible to circumvent their antigenicity – either by chemically modifying them or by making use of small active fragments – it should also be possible to introduce them into therapy. Thus, they would eventually become as important as the low molecular weight plant constituents,

in the shadow of which plant proteins at present stand.

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