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Soluble Lectins: A New Class of Extracellular Proteins

Samuel H. Barondes

Lectins are carbohydrate-binding proteins that were originally identified in plant extracts as agglutinins of erythrocytes (*1*). They have two critical properties: specificity for particular sugar residues; and bivalency or polyvalency (*2*). These properties make them useful for many purposes, including blood typing and the purification of glycoconjugates. For years they found wide applications as reagents, and little thought was given to the role they play in the plants from which they were derived.

Summary. Soluble lectins of cellular slime molds and vertebrates are present at extracellular sites in the developing or adult tissues that make them. Some lectins are concentrated around cell groups, as in extracellular matrix or elastic fibers. Others are at the interface between cells and the external environment, as in mucin or slime. Specific glycoproteins, proteoglycans, or polysaccharides that bind these endogenous lectins may also be present at these sites. Interactions between the lectins and glycoconjugates appear to play a role in shaping extracellular environments.

In the past decade, interest in the functions of lectins has increased considerably. This was stimulated by their isolation from many other organisms, including vertebrates and cellular slime molds—thereby attracting the attention of a wide range of biologists. In this article I summarize work on soluble lectins derived from these newer sources, especially as it relates to their biological roles in the tissues that make them. Studies of the functions of lectins in plants have been reviewed (*3*), and their relevance is considered briefly at the end of the article.

Soluble and Membrane Lectins

Lectins can be subdivided on the basis of whether or not they are integrated into membranes. The integral membrane lectins require detergents for solubilization, but the soluble ones do not. This subdivision probably reflects a fundamental difference in the general functions of these classes. In this view, the integral membrane lectins appear to have evolved to bind glycoconjugates to membranes, either at the cell surface or within vesicles. This results in the localization of the glycoconjugates at particular membrane sites or their transport to other cellular compartments (*4*). In contrast, soluble lectins, being excluded from membranes, cannot directly function in this way. Instead, they can move freely in the aqueous compartments within and between cells, interacting with both soluble and membrane-bound glycoconjugates.

The concept that integral membrane lectins function in endocytosis of glycoproteins or their intracellular translocation has experimental support (*4*). For example, the lectin of mammalian liver membranes that binds galactose is believed to function as a cell surface receptor for circulating asialoglycoproteins that contain terminal galactose residues. The asialoglycoprotein-lectin complex on the hepatocyte surface then undergoes endocytosis and is transported to other cellular compartments; this process may lead to degradation of the glycoprotein (*4*) or replacement of sialic acid (*5*). Several other vertebrate carbohydrate-binding proteins have been identified and these also appear to function in cellular translocation of glycoproteins (*4*). They bind other carbohydrate residues including mannose 6-phosphate, fucose, *N*-acetylglucosamine, and mannose. The functions of these integral

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membrane lectins have been reviewed (*4*) and are not considered further here (*6*).

In contrast, the function of the soluble lectins has been more difficult to infer, partly because these lectins tend not to be as sharply localized as those in membranes. However, immunohistochemical and developmental techniques have provided clues to the endogenous functions of soluble lectins in several experimental systems. One recurrent finding in studies with both vertebrates and cellular slime molds is that these proteins, which may initially be concentrated inside cells, are ultimately externalized. This suggests that a common function of soluble lectins is to bind to the complementary glycoconjugates on and around the cells that release them. Since both the lectins and glycoconjugates may be multivalent, large macromolecular complexes that shape extracellular environments may be formed.

Soluble Vertebrate Lectins

Most of the soluble lectins isolated from vertebrate tissues (Table 1) bind β-galactosides. The best studied are a group of dimeric proteins found in many organisms, including the electric eel (*7*), calf (*8*), chicken (*9*), rat (*10*), and man (*10, 11*). These proteins share not only similar subunit molecular weights and a dimeric structure, but also a requirement for a reducing agent to maintain their carbohydrate-binding activity. A dimeric β-galactoside–binding protein has also been found in snake venom (*12*), but it differs from the group under discussion in requiring calcium ion for hemagglutination activity and in being inhibited by reducing agents.

Another group of vertebrate β-galactoside–binding lectins can be isolated as monomers. The one examined in most detail was purified from chicken intestine (*13*) and is designated chicken lactose–lectin-II (CLL-II). It differs from the dimeric chicken lactose–lectin-I (CLL-I) in subunit molecular weight, isoelectric point, and peptide map; it is also immunologically distinct. Since CLL-II can agglutinate erythrocytes by binding to their surface carbohydrates, it must either have two carbohydrate-binding sites per monomer or be capable of some degree of dimerization. A monomeric β-galactoside–binding lectin isolated from rabbit bone marrow has been named erythroid developmental agglutinin (*14*).

Other soluble vertebrate lectins are multimeric. The serum of the eel *Anguilla rostrata* contains a lectin which has 12 subunits per molecule and is the first vertebrate lectin to have been characterized (*15*). A soluble lectin from *Xenopus laevis* oocytes and embryos also contains about 12 subunits per molecule (*16*). Other frog eggs have different lectins (*17, 18*) not yet fully characterized.

Another lectin, found in both chicken (*19*) and rat (*20*) tissues, reacts well with heparin and related glycosaminoglycans. The lectins in these two species appear to be similar, and their tendency to form large aggregates complicates their study. Fibronectin (*21*) and laminin (*22*), major extracellular glycoproteins, also bind heparin (*23*) and agglutinate erythrocytes (*24*). Both might have been classified as lectins, had they been isolated in a search for erythrocyte agglutinins; and both may have functional similarities with the lectins under consideration.

Peak Levels in Embryonic or Adult Tissues

A property shared by many vertebrate lectins is that they are maximally synthesized at specific stages in the embryonic development of certain tissues (*25–31*). For example, CLL-I levels in embryonic muscle become maximal as it differentiates (*25–27*) and decline thereafter (Fig. 1). Likewise, CLL-II levels in liver (Fig. 1) and kidney are much higher in the embryo than the adult (*27*). Rat β-galactoside–binding lectin peaks during myoblast differentiation (*25, 28*) and lung development (*29*). Erythroid developmental agglutinin is found in erythroblasts but not mature erythrocytes (*14*), and chicken heparin lectin is most highly concentrated in embryonic muscle (*30*).

However, in some tissues, these lectins may reach peak levels only in the adult. For example, CLL-I is scarce in embryonic liver but plentiful in adult liver (*13, 27*); CLL-II becomes prominent in intestine only late in development, and remains at high levels thereafter (Fig. 1) (*27*). These results suggest that the same lectin that is synthesized for developmental roles in some tissues may also be used in others to mediate adult properties.

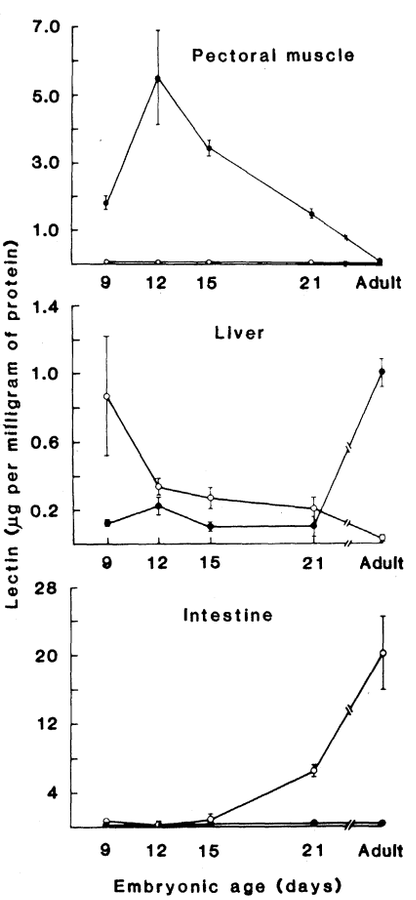

Fig. 1. Chicken lactose–lectin-I (●) and chicken lactose–lectin-II (○) in pectoral muscle, liver, and intestine. Lectin concentrations were determined by radioimmunoassay (*27*) in extracts of tissues derived from embryos of the indicated ages, and from adults.

Immunohistochemical Studies Suggest Secretion

Important clues to lectin function come from immunohistochemical studies with antibodies to the purified proteins, which reveal the distribution of the endogenous lectins in these tissues. Such studies should not be confused with those in which labeled lectins from other sources are added to tissues for the purpose of locating the glycoconjugates that bind them, a method that has also been used to identify the potential receptors for endogenous lectins (as discussed below).

A major finding in these studies is that soluble vertebrate lectins appear to be secreted. In some cases, there is a shift from an intracellular to an extracellular location with differentiation. For example, CLL-I is concentrated intracellularly in developing chicken muscle but extracellularly with maturation (Fig. 2) (*32*). Its apparent secretion from polynucleated myotubes argues against the suggestion (*28, 33*) that the lectin participates in an earlier process, myoblast fusion, as do other studies (*34*). Instead, its secretion into extracellular matrix (Fig. 2) suggests that it may function by interacting with glycoconjugates at this site. There is also evidence that chicken heparin lectin is secreted by muscle cells differentiating in tissue culture (*35*).

Soluble lectins may also be secreted

by adult tissues. This was first suggested by immunohistochemical studies with the electron microscope that showed CLL-I localized extracellularly around the acini of adult pancreas (*36*). Rat β-galactoside lectin is also secreted, in this case to a specialized extracellular site, elastic fibers (Fig. 3) (*37*). The lectin is found there in both mature pulmonary alveoli and capillaries (Fig. 3). Adult lectin secretion is also suggested by the localization of CLL-II in the secretory granules of the goblet cells and on the mucosal surface (Fig. 4), and this is confirmed by physiological studies (*38*).

The immunohistochemical location of *Xenopus* lectin suggests that it too is secreted (*39*). In oocytes and unfertilized eggs, the lectin is concentrated intracellularly—some in the cortical granules, but most associated with the yolk platelets (*39*). With fertilization, the cortical granules discharge their contents, and it has been proposed that the released lectin reacts with glycoconjugates around the egg (*40*). In blastula stage embryos, the lectin seems to collect in the extracellular cleavage furrows of the embryo (*39*), suggesting that it may function by binding to glycoconjugates in the matrix between the dividing cells.

Lectins in Cellular Slime Molds

Isolation of lectins from cellular slime molds (*41*) has facilitated studies of the endogenous role of such proteins, because these primitive eukaryotes have many favorable properties for biological investigations (*42*). The most important is that they can be readily induced, by starvation, to differentiate from a unicellular to a multicellular form. The individual amoeboid cells, which contain no detectable lectins, synthesize them in large quantities as they form aggregates and develop into slugs, spores, and stalk.

With differentiation, there are about 5×10^6 lectin molecules per cell, constituting several percent of the total soluble protein. The lectins from all species of cellular slime molds studied have subunit molecular weights in the range of 25,000, and all bind β-galactosides to some degree, although their pattern of binding to a series of other sugars varies (*43*). *Dictyostelium discoideum*, the most extensively studied of this group, synthesizes two lectins, discoidin I and discoidin II (*44*). Both are tetrameric proteins but they are readily distinguished by subunit molecular weight, isoelectric point, and peptide map (*44*). Antibodies to them may show a small amount of cross-reaction (*45, 46*), but, for practical purposes, they are immunologically distinct. Both bind β-galactosides, but discoidin I has a much higher affinity for *N*-acetylgalactosamine (*47*). Synthesis of the two lectins is also regulated differently (*44, 48, 49*). Discoidin I levels become maximal as aggregation is completed, and decline thereafter; discoidin II levels become maximal as fruiting body formation progresses (*49*) (Fig. 5). This is one indication that these lectins have different functions.

Because discoidin I is synthesized as cell-cell adhesion develops (*41, 50*), its possible role in this process has been considered (*51*). Adhesion might result by lectin binding to glycoconjugates on

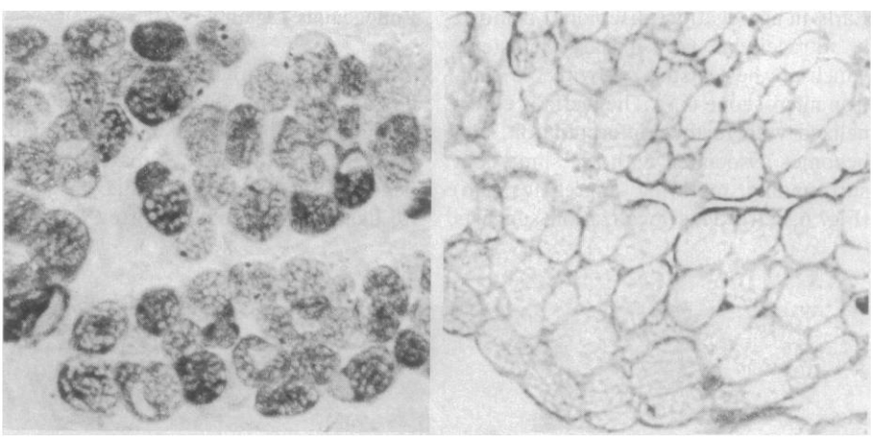

Fig. 2. Externalization of chicken lactose–lectin-I with maturation of pectoral muscle. Lectin was immunohistochemically located by the binding of rabbit antibodies directed against it in sections from a 19-day embryo (left) or from a 2-day-old chick (right). Bound antibody was then reacted with goat antiserum to immunoglobulin coupled to peroxidase. Lectin is indicated by the dark reaction product of a peroxidase detection procedure (*32*). It is concentrated within polynucleated muscle cells in the embryo, but 4 days later, in the 2-day-old chick, it is mostly extracellular (×2200).

Table 1. Soluble vertebrate lectins.

Designation	Major sources	Subunit (molecular weight)	Subunits per molecule	Ligand	References
Electrolectin	*Electrophorus electricus* electric organ	16,500	2	β-Galactoside	(*7*)
Calf β-galactoside lectin	Heart, lung, spleen, muscle	13,000	2	β-Galactoside	(*8*)
Chicken lactose–lectin-I	Embryonic muscle, adult liver	15,000	2	β-Galactoside	(*9*)
Rat β-galactoside lectin	Lung	13,500	2	β-Galactoside	(*10*)
Human β-galactoside lectin	Lung	14,000	2	β-Galactoside	(*10, 11*)
Thrombolectin	*Bothrops atrox* (snake) venom	15,000	2	β-Galactoside	(*12*)
Chicken lactose–lectin-II	Intestinal mucosa	14,000	1	β-Galactoside	(*13*)
Erythroid developmental agglutinin	Rabbit bone marrow	13,000	1	β-Galactoside	(*14*)
Anguilla rostrata (eel) lectin	Serum	10,000	12	L-Fucose	(*15*)
Xenopus laevis lectin	Egg, embryo	43,000; 45,000	~ 12	α- or β-Galactoside	(*16*)
Rana catesbiana lectin	Egg	210,000	?	β-Galactoside	(*17*)
Rana japonica lectin	Egg	13,500	?	Glycoprotein rich in sialic acid	(*18*)
Chicken heparin lectin	Embryonic muscle, adult liver	13,000; 16,000	?	Heparin, *N*-acetylgalactosamine	(*19*)
Rat heparin lectin	Lung	13,000; 16,000	?	Heparin, *N*-acetylgalactosamine	(*20*)

the surfaces of adjacent cells. Isolation of mutants that appeared to synthesize functionally impaired discoidin I, and that had diminished cell adhesion and aborted development, supported this possibility (*52*). However, the fact that antibodies to the lectin do not block adhesion in an in vitro assay (*53*) argues against it. Evidence for participation of endogenous lectins in cell-cell adhesion of another cellular slime mold, *Polysphondylium pallidum*, is also considerable (*54*), but subject to alternative interpretations (*51*).

Location of Discoidins I and II by Immunohistochemistry

As with the vertebrate lectins, ascertaining the location of discoidin I and II has provided clues to their functions. Early in aggregation, discoidin I is mostly intracellular (*46*) in large clusters, which are best visualized with the electron microscope (*55*). The lectin is externalized with further differentiation, and becomes associated with the slime coat surrounding maturing aggregates (*46*) (Fig. 6, left). This localization suggests that discoidin I functions extracellularly by interacting with slime coat glycoconjugates, rather than as a direct cell-cell ligand.

Discoidin II is first found in a wavy distribution within aggregates, suggesting an intercellular localization (*46, 49*). Later, more accumulates (Fig. 5) in a punctate distribution in cells that are differentiating into spores (Fig. 6, right). Its distribution overlaps with that of antigens localized in prespore vesicles (*46, 49*). The discoidin II is then externalized around the differentiating spore cells; but it is not detectable around mature spores (*46, 49*). These results suggest that discoidin II is secreted along with the polysaccharide that forms the spore coat (*47*) and may, therefore, interact with it, both inside and outside the cell.

Endogenous Ligands

To determine the functions of lectins, it is necessary to isolate the tissue glycoconjugates which they normally bind. Thus far, only a few such materials have been identified. Their candidacy as natural ligands has been supported not only by their abundance in tissues rich in the lectin but also by their localization in the same tissue compartment. For example, intestinal mucin, a highly glycosylated glycoprotein that contains many terminal β-galactoside residues, appears to be an endogenous ligand for CLL-II (*38*). Both are found in the secretory granules of the goblet cells and on the intestinal mucosal surface; and the mucin and lectin bind well to each other. A glycosaminoglycan appears to be an endogenous ligand for chicken heparin lectin not only because they bind to each other but also because both are externalized by differentiating primary muscle cultures (*35*).

Similar evidence suggests that a polysaccharide synthesized late in *D. discoideum* development is an endogenous ligand for discoidin II (*47*). This material comprises about 5 percent of the carbohydrate residues of the organism at this stage and becomes associated with the spore coat. It contains mostly galactose and *N*-acetylgalactosamine and binds well to discoidin II when measured in a competitive binding assay. Immunohistochemical studies suggest that both discoidin II and the polysaccharide are localized in vesicles in prespore cells, and are secreted around these cells (*49*). A macromolecule that associates with discoidin I in the slime that coats early aggregates appears to be an endogenous ligand for this lectin (*49*). It is not well characterized but has very little affinity for discoidin II.

Localization studies in which a lectin derived from a given tissue is added to sections of that tissue has also been used to identify possible endogenous receptors. For example, discoidin I and discoidin II have been added to sections of *D. discoideum* aggregates fixed in a manner that destroys the antigenicity of the endogenous lectin but preserves the lectin binding activity of the endogenous ligand (*49*). These conditions are not difficult to achieve since protein antigens are sensitive to many denaturants that do not affect glycoconjugates. The location of the added bound lectin can then be determined immunohistochemically.

Added discoidin I binds well to the slime coat surrounding maturing aggregates, whereas discoidin II does not. This indicates that a ligand specific for discoidin I is localized at this site, like the endogenous lectin itself. The polysaccharide ligand for discoidin II is less specific in that it also binds somewhat to discoidin I, both in sections (*49*) and after isolation (*47*). Despite this cross-reactivity, this polysaccharide probably interacts normally only with discoidin II,

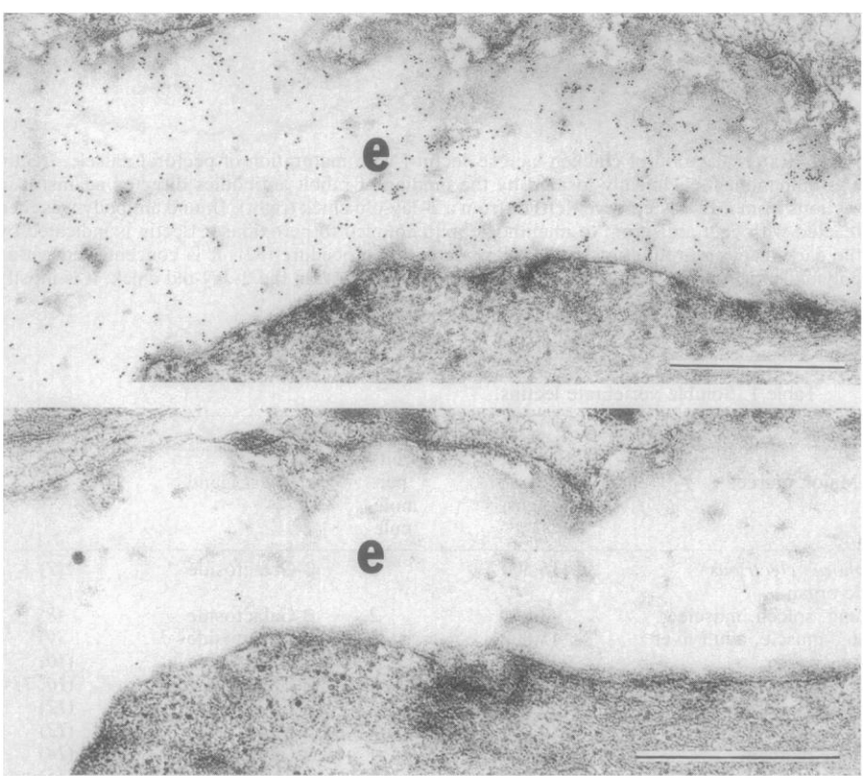

Fig. 3. Localization of rat β-galactoside lectin in elastic fibers of a pulmonary blood vessel. Lectin was immunohistochemically located (*37*) by on-grid staining with an affinity purified rabbit immunoglobulin G7 (IgG) to lectin (top), and the bound antibody was visualized by reaction with goat antiserum to rabbit IgG complexed to colloidal gold. The large pale zone of elastic fibers (*e*) is heavily stained with small black particles of colloidal gold in the section reacted with antibody to lectin (top) but not in the section reacted with control immunoglobulin (bottom) (bar, 0.1 μm).

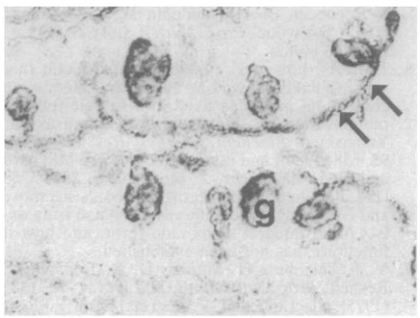

Fig. 4. Chicken lactose–lectin-II in sections of adult intestine. Lectin was immunohistochemically located with a specific antiserum (*38*) and is indicated by the dark reaction product of a peroxidase detection procedure, as in Fig. 2. It is prominent in goblet cells (*g*), where it is concentrated in clusters which are secretory granules, and at the mucosal surface (arrows) (×1000).

since only that lectin is found with it in and around differentiating spore cells. Discoidin I could also interact with the polysaccharide, but their disparate temporal and spatial expression precludes this. A biologically meaningful interaction will result only if participants of appropriate complementarity are at the right place at the right time. Similar studies with CLL-I distinguish between natural ligands with which it co-localizes and other potential ligands in tissue sections not normally found with the lectin (*56*).

One characteristic of mucin, spore polysaccharide, and heparin is that they are large glycoconjugates that may each bind more than one lectin molecule. The polyvalency of these ligands could stabilize their interaction with the divalent or polyvalent lectins, even if individual glycoconjugate residues bound with low affinity. In this regard, it is important to recognize that integration of univalent glycolipids or glycoproteins into membranes renders them functionally polyvalent, since they can form clusters by moving together within the plane of the membrane. Lectin could then bind well to such clusters, even though individual interactions had a low affinity. Yet, it would be difficult to identify such ligands when solubilized from membranes, since binding in the univalent state would be so weak. It is not known if functionally significant ligands have been overlooked because of this problem. Vertebrate and slime mold lectins can certainly bind ligands in cell surface membranes in a biologically effective way, since they induce cell division of B lymphocytes (*57*).

Plant Lectins

Recent work with plant lectins suggests that they, too, may be externalized and function as extracellular proteins. For example, there is evidence that many plants contain lectins on and around root surfaces (*3*, *58*). Such externalization is analogous to that of CLL-II, which associates with glycoconjugates in the intestinal lumen (*38*), likewise a site external to the organism. The localization of discoidin I around aggregates (*46*, *49*) that would normally form in soil is also analogous.

Plant lectins have also been demonstrated in stem and leaf tissues not normally associated with the soil. Very little is known about these materials, but Etzler (*3*) has reviewed some evidence that they are associated with cell walls. Such an association would be analogous to the localization of CLL-I (*32*, *36*), and rat β-galactoside lectin (*37*) at extracellular sites between cells within the organism.

Conclusion

Immunohistochemical studies show that cells frequently secrete their soluble lectins and that these proteins may remain near the cells that make them. In this sense, then, these soluble lectins may be viewed as a class of extracellular proteins. Since extracellular materials are rich in glycoconjugates and since, in some cases (including mucin, heparin, spore polysaccharide), they have been shown to bind to an endogenous lectin in vitro, it is likely that such binding occurs in nature and is biologically significant.

Like well-known extracellular proteins, such as collagen and fibronectin, some lectins may participate in the organization of extracellular matrix. Many of the matrix proteins and proteoglycans accumulate at specific times in development (*59*), like some of the lectins discussed in this article. This suggests that both groups may participate in the organization of the cellular environment, and thereby influence morphogenesis. Since

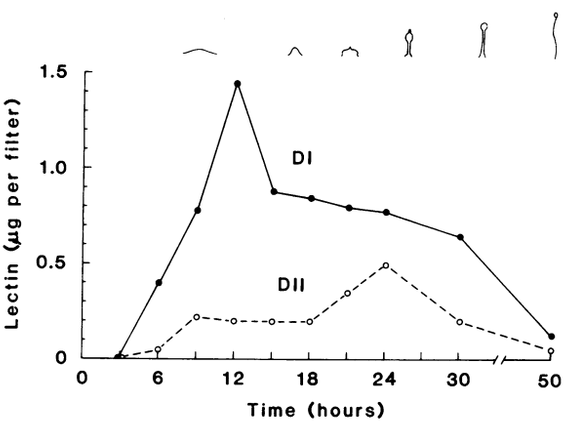

Fig. 5. Discoidin I (●) and discoidin II (○) in differentiating *D. discoideum*. About 5×10^7 cells were layered on each of a group of moist filters. Many aggregates formed on each filter and their typical morphology at a given time is sketched at the top. The amount of each lectin in the entire contents of each filter was determined by means of highly specific immunoassays (*49*).

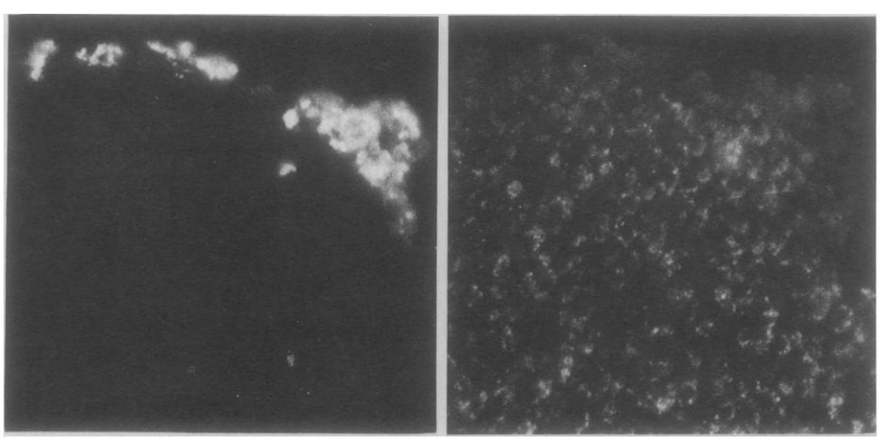

Fig. 6. Immunohistochemical location of discoidin I (left) and discoidin II (right) in a late aggregate of *D. discoideum*. A section of the aggregate was stained with rabbit antibody to discoidin I and mouse antibody to discoidin II and the bound antibodies are visualized by specific fluorescence microscopy after reaction of the sections with fluorescein-labeled goat antiserum to rabbit IgG and rhodamine-labeled goat antiserum to mouse IgG (*46*). The field shown contains about 10^2 cells. Discoidin I is localized around the aggregate, whereas discoidin II is largely in small clusters within the cells (×2000).

lectins and their ligands may both have multiple binding sites, their interactions could lead to the formation of viscoelastic gels. These could give rise to properties of the developing extracellular matrix that regulate both cell migration and adhesion. The high concentration of rat β-galactoside lectin in elastic fibers of lung and blood vessels suggests a possible role in tissue elasticity.

The soluble lectins are not, however, confined to extracellular matrix. In the chicken intestine, they are secreted into a very different extracellular locale, the mucosal surface. They may also function intracellularly, possibly in the process of glycoconjugate secretion. In some instances, their role may not be to hold glycoconjugates together but to keep them apart. Thus, the lectin-glycoconjugate interactions in secretory granules—as with discoidin II in prespore cells and CLL-II in intestinal goblet cells—may block glycoconjugate-glycoconjugate interactions prior to secretion. When they are released from the cells, the glycoconjugates may associate through carbohydrate-carbohydrate interactions into complex structures that might exclude the lectins.

Despite considerable progress, we are just beginning to understand the functions of the soluble lectins. Since a given lectin can bind to different glycoconjugates, it may participate in many biologically significant interactions with cell surface glycoproteins and glycolipids as well as with soluble glycoproteins and polysaccharides. A fuller understanding of a specific case will require identification of all the glycoconjugates with which the lectin actually associates in a particular tissue, and at a given developmental stage.

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