Journal of Supramolecular Structure
and Cellular Biochemistry 16:233-242 (1981)
Cellular Recognition 227-236

Endogenous Lectins in Chickens and Slime Molds: Transfer From Intracellular to Extracellular Sites

Samuel H. Barondes, Eric C. Beyer, Wayne R. Springer, and Douglas N. Cooper

Department of Psychiatry, University of California, San Diego, La Jolla, California 92093 and Veterans Administration Medical Center, San Diego, California 92161

Endogenous lectins in both cellular slime molds and chicken tissues have been localized primarily intracellularly, in contrast with the predominantly extracellular localization of the glycoproteins, glycolipids, and glycosaminoglycans with which they might interact. Here we present evidence that lectins in both of these organisms may be externalized and become associated with the cell surface and/or extracellular materials. In chicken intestine, chicken-lactose-lectin-II is shown to be localized in the secretory granules of the goblet cells, along with mucin, and to be secreted onto the intestinal surface. In embryonic muscle, chicken-lactose-lectin-I is shown to be externalized with differentiation, ultimately becoming localized on the surface of myotubes and in the extracellular spaces. In a cellular slime mold, Dictyostelium purpureum, externalization of lectin is elicited by either polyvalent glycoproteins that bind the small amount of endogenous cell surface lectin, or by slime mold or plant lectins that bind unoccupied complementary cell surface oligosaccharides. These results suggest that externalization of endogenous lectin may be a response to specific external signals. We conclude that lectins are frequently held in intracellular reserves awaiting release for specific external functions.

Key words: lectins, slime mold lectins, vertebrate lectins, chicken-lactose-lectin-I, chicken-lactose-lectin-II, chicken heparin lectin, Dictyostelium, secretion, muscle development, extracellular materials

Lectins are defined as a class of carbohydrate-binding proteins that are not products of the immune system and that have no known enzymatic activities. Although most extensively studied in plant seeds, many lectins have recently been found in other organisms including vertebrate tissues. This wide distribution has stimulated interest in the function of lectins in the cells that make them [1].

One approach to determining the endogenous role of lectins is to localize them by immunohistochemical and biochemical techniques. The purpose of this report is to describe recent studies of the distribution of lectins in chicken tissues and cellular slime molds. In both systems endogenous lectins are found to be intracellular [1]. In contrast, many of the glycoproteins, glycolipids, and glycosaminoglycans with which they might interact are localized on the cell surface or extracellularly. In the present report we summarize some studies indicating that, under certain circumstances, lectins are externalized and become associated with the

Received May 11, 1981; accepted May 25, 1981.

0275-3723/81/1603-0233$03.00

cell surface and/or extracellular materials. Based on this and other evidence, we conclude that endogenous lectins are often held in an intracellular reserve, then externalized, presumably to make them available to substances with which they can interact. After externalization they apparently perform biologically significant functions by binding complementary complex heterosaccharides on the cell surface, in the surrounding glycocalyx, or at more distant extracellular sites.

LECTIN EXTERNALIZATION IN CHICKEN TISSUES

Properties of Endogenous Chicken Lectins

Two types of lectins have been purified from chicken tissues. One type requires detergent for solubilization, suggesting that such lectins are integral membrane proteins. A lectin of this type is believed to play a role in the uptake of partially degraded circulating glycoproteins by hepatic parenchymal cells [2]. The other type of lectin can be isolated in soluble form without detergent. Such lectins are not integrated into membranes, although they may be bound to the saccharide components of membrane glycoproteins or glycolipids. Lectins of this second type are the subject of this paper. Three such lectins have been purified from chicken tissues [3–6]. Two of these lectins bind lactose and are called chicken-lactose-lectin-I (CLL-I) and chicken-lactose-lectin-II (CLL-II) [5]. The other interacts strongly with heparin, but also shows a specific interaction with a simple sugar, N-acetyl-D-galactosamine [6]. It is referred to as chicken-heparin-lectin (CHL).

All three soluble chicken lectins are found in more than one tissue [5–7], where their levels may vary strikingly at different stages of differentiation. For example, CLL-I is very prominent in developing pectoral muscle, and becomes virtually undetectable in the adult [7]. In contrast, the level of CLL-I in liver is much higher in the adult than in the embryo [7]. CLL-II is relatively highly concentrated in embryonic kidney, falling to much lower levels in the adult [7]. In contrast, it is at its highest levels in adult intestine [7]. CHL has been purified from embryonic muscle [6], where it undergoes changes with development [8]. It is also present at high levels in adult chicken liver [6]. A very similar lectin has recently been purified from rat lung [9].

The function of these three soluble chicken lectins is not yet clearly understood. Based on the circumstances of their developmental expression and localization, they appear to play different roles in different tissues and at different stages of development [1]. In the present report we will not be concerned primarily with the endogenous functions of these lectins, but rather with evidence that all three may be externalized.

Evidence for Lectin Externalization in Chicken Tissues

The most extensive evidence for externalization of endogenous chicken lectins has been obtained in studies of the intestine. Using antibodies that are specific for CLL-II, and that do not cross-react with CLL-I or other proteins of chicken intestinal extracts [7], CLL-II has been localized to the secretory vesicles of the goblet cells of the chicken intestine (Fig. 1). Some lectin is also detectable on the mucosal surface of the intestine (Fig. 1). The lectin in the secretory vesicles is presumably associated with intestinal mucin, to which it can bind [10]. It is therefore inferred that CLL-II is secreted from these vesicles into the intestinal lumen in as-

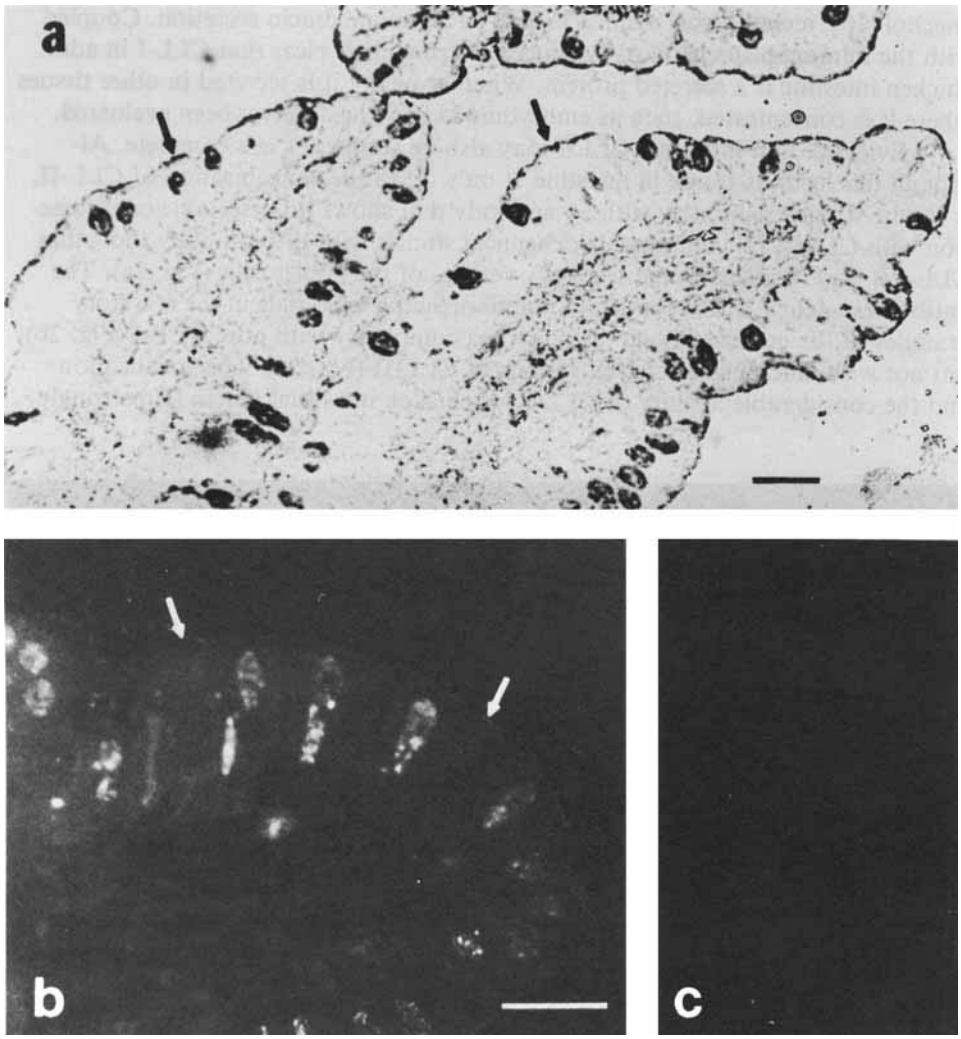

Fig. 1. Localization of chicken-lactose-lectin-II in sections of adult chicken intestine. Thin frozen sections of fixed intestine were reacted with rabbit antibody raised against highly purified CLL-II. Antibody binding was localized either with the peroxidase-anti-peroxidase method or with rhodamine labeled goat antirabbit IgG. a) Peroxidase-anti-peroxidase method. b) Fluorescence method. c) control for (b) in which the primary antibody was adsorbed with boiled highly purified CLL-II. The reaction product in (a) and (b) is localized in the secretory granules of the goblet cells and on the surface of the intestinal villi (arrows). Bar equals 10 μm. For details see [10].

sociation with mucin, and that it plays some role in the organization of the mucin during its secretion and/or its association with the intestinal mucosal surface. In this case, then, the externalization process appears to proceed through classical secretion in which products stored in vesicles are released by vesicle fusion with the plasma membrane.

Direct studies of CLL-II secretion by intestinal mucosal cells support this hypothesis. As would be expected, some lectin can be eluted from the surface of the intestinal mucosa with lactose [10]. This amount is significantly increased by beth-

anechol [10], a cholinergic agonist known to stimulate mucin secretion. Coupled with the immunohistochemical evidence, it seems fairly clear that CLL-I in adult chicken intestine is a secreted protein. Whether or not it is secreted in other tissues where it is concentrated, such as embryonic kidney, has not yet been evaluated.

Evidence that intestinal CLL-I may also be secreted is less complete. Although this lectin is found in intestine at only 0.5% the concentration of CLL-II, it can be studied separately with an antibody that shows no detectable cross-reaction with CLL-II [7]. Immunohistochemical studies with this antibody show that CLL-I is also localized in the secretory vesicles of the goblet cells (Fig. 2a). The antibodies against CLL-I that stain immunoreactive materials in the secretory granules of the goblet cells can be adsorbed completely with pure CLL-I (Fig. 2b), but not with much higher concentrations of CLL-II (Fig. 2c). This localization and the considerable affinity of CLL-I for chicken intestinal mucin [10] strongly

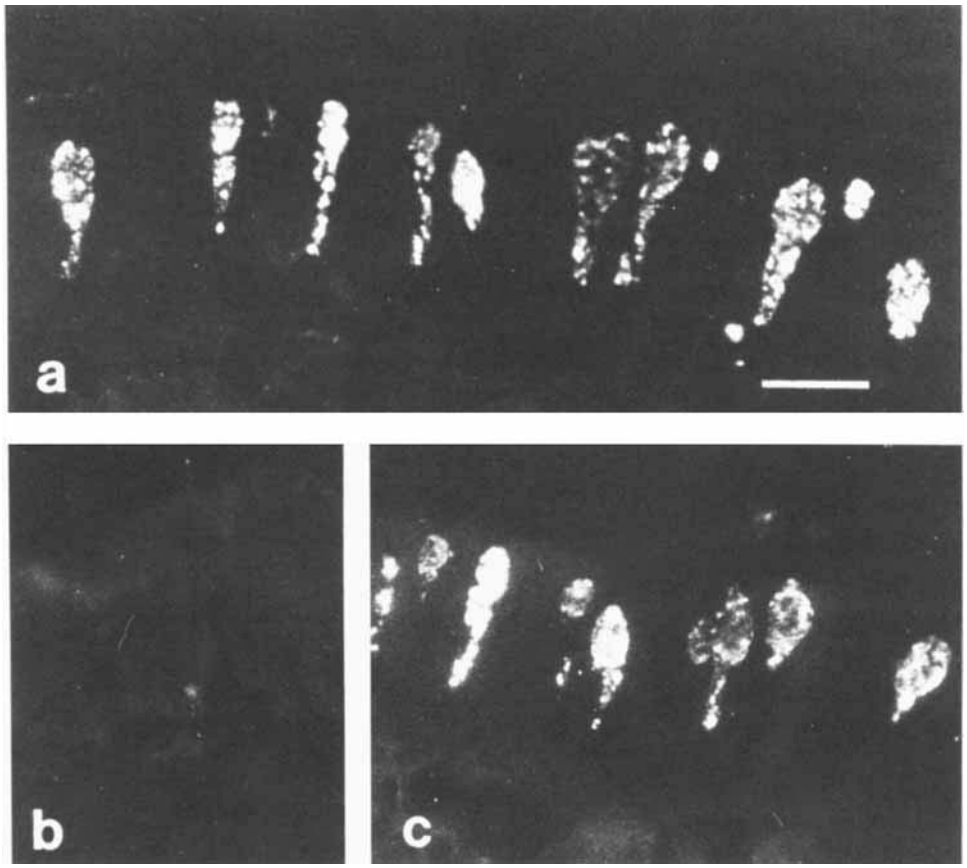

Fig. 2. Localization of chicken-lactose-lectin-I in sections of adult chicken intestine. Thin frozen sections of fixed intestine were reacted with rabbit antibody raised against CLL-I followed by reaction with rhodamine-labeled goat antirabbit IgG. a) Primary antibody was used without preadsorption. b) Primary antibody was adsorbed with boiled purified CLL-I. c) Primary antibody was adsorbed with boiled purified CLL-II, at ten times the concentration of the purified CLL-I used in (b). Bar equals 10 μm. For details see [10].

suggest that it, too, is secreted in this tissue. However, unlike CLL-II, immunohistochemical localization of CLL-I on the intestinal mucosal surface and evidence for its secretion by bethanechol have not been achieved, possibly because of its much lower concentration in this organ [7, 10].

Immunohistochemical studies also suggest that CLL-I in embryonic muscle is externalized [11]. In relatively undifferentiated muscle cells the lectin is intracellular in a fairly diffuse pattern (Fig. 3a). However, with further development, CLL-I is found in a pattern suggesting that it is associated with the intracellular tubular systems that run parallel and perpendicular to the long axis of the myotubes (Fig. 3c). Based on immunohistochemistry with the light microscope, it is not possible to tell whether the lectin is on the cytoplasmic side of this tubular network or within the lumen of the tubules. The latter seems likely since, late in muscle development, the lectin becomes localized on the surface of the myotubes and in the extracellular space (Fig. 3d, e). Taken together, these studies suggest that CLL-I migrates from its site of synthesis into the lumen of the intracellular tubular systems and then extracellularly. The initial step in externalization is presumed to occur by passage of lectin through the membranes of the sarcoplasmic reticulum. Movement to the cell surface apparently does not require fusion of vesicles with the plasma membrane. Instead, the localization studies suggest [11] that lectin migrates directly to the myotube surface via an intracellular tubular system, which is continuous with the plasma membrane. This is a different type of externalization than the vesicular secretion found in intestine, but the result, externalization, appears similar in both tissues.

CLL-I has also been found at an external site, the extracellular space, in adult pancreas, by immunofluorescence and immunoelectron microscopy [12]. The process by which the lectin is transported to this position has not been studied. Since a number of other tissues contain binding sites for CLL-I in cell surfaces, extracellular spaces, and basement membranes [13], externalized lectin may participate in many types of interactions.

Studies with CHL in developing muscle cultures also indicate externalization [14]. This lectin, which is prominent in developing muscle in vivo, is absent from the medium of chicken myoblasts and early myotubes raised in culture [14]. However, after considerable myoblast fusion has occurred, CHL becomes abundant in the medium [14]. These results suggest processing like that of CLL-I. Unfortunately, specific antibodies for CHL have not yet been obtained, so that its histological localization is not known.

LECTIN EXTERNALIZATION IN CELLULAR SLIME MOLDS

Cellular slime molds are a class of organisms that have received increasing attention because they are extremely useful for studies of development and morphogenesis. They rapidly differentiate from an amoeboid form characteristic of their vegetative state to an aggregating colonial form within hours after starvation. One concomitant of this differentiation is the marked synthesis of lectins, which are present at very low or undetectable levels in vegetative cellular slime molds, but which comprise several percent of the protein of aggregating slime molds [15, 1]. Like chickens, cellular slime molds contain more than one lectin. In one species, Dictyostelium purpureum, seven lectins have been purified and char-

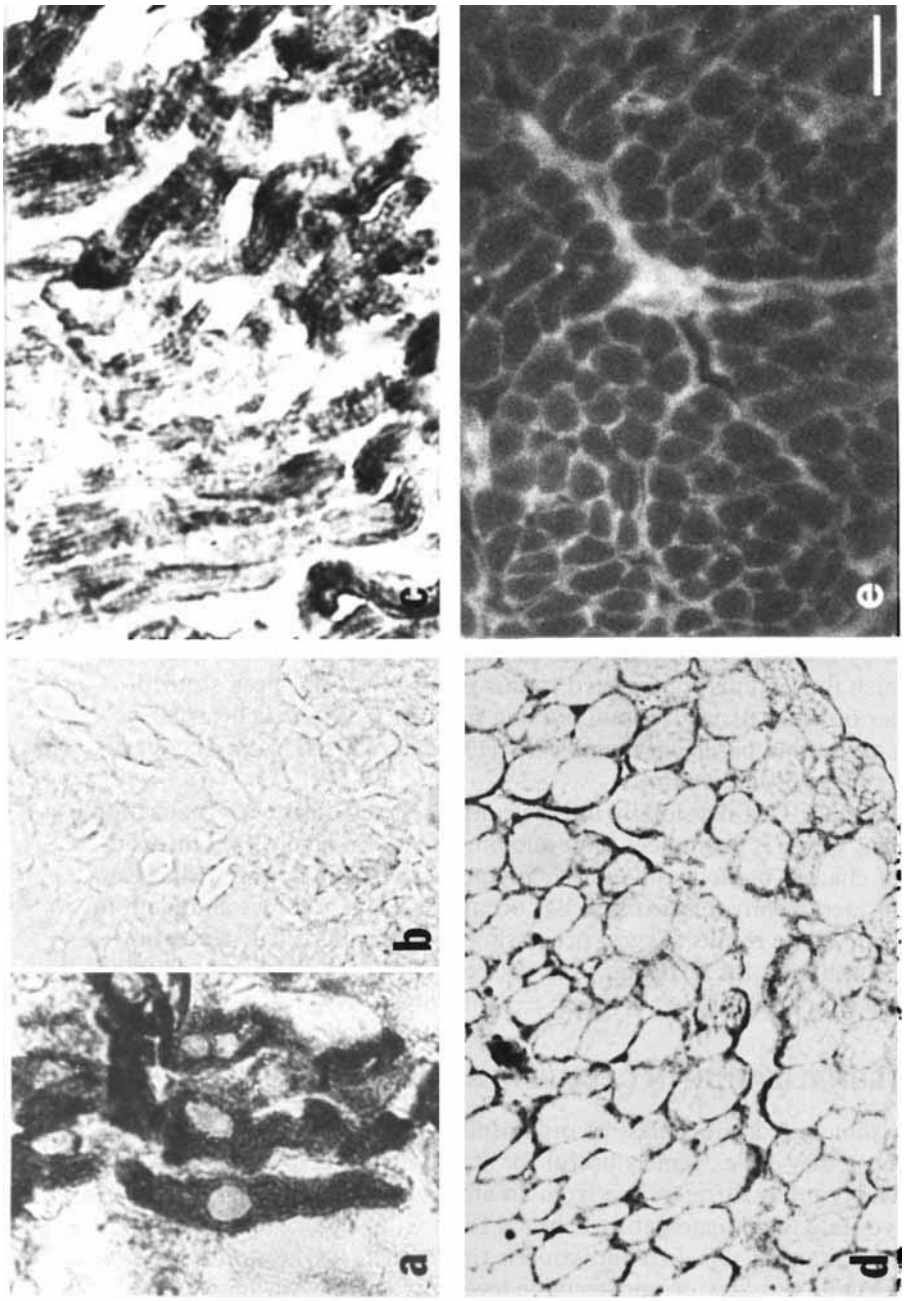

Fig. 3. Localization of chicken-lactose-lectin-I in chicken pectoral muscle at several developmental stages. Thin frozen sections of fixed chicken pectoral muscle were prepared at various stages of development and reacted with rabbit antibody raised against CLL-I. Antibody binding was visualized either by the peroxidase-anti-peroxidase method or by indirect staining with rhodamine-labeled goat antirabbit IgG. a, b) Material from 10-day-old chick embryo studied with the peroxidase-anti-peroxidase method using anti-CLL-I in (a) and anti-CLL-I adsorbed with boiled purified CLL-I in (b). c) Longitudinal section from 17-day-old chicken embryo, reacted with antibody to CLL-I that was visualized with the peroxidase-anti-peroxidase method. d, e) Cross sections of pectoral muscle from chickens 2 days after hatching reacted with antibody to CLL-I and visualized either by the peroxidase-anti-peroxidase method (d) or with rhodamine-labeled goat antirabbit IgG. Bar equals 5 μm. For details see [11].

acterized [16]. They are made from at least four gene products. Five of the lectins are the five possible tetramers that can be formed by random association of two of the subunits. The other two are each homotetramers of the remaining two types of subunit [16]. Whereas the role of lectins in cellular slime molds is not clearly understood, there is evidence that they may function in the development of cell-cell adhesion, during differentiation. Much of the evidence for this hypothesis is circumstantial, and has been reviewed recently [1].

If slime mold lectins are important for cell-cell adhesion, it might be expected that they would be concentrated on the cell surface. However, careful studies of the distribution of lectins in aggregating D purpureum cells indicate that only about 2% is present at this site [17]. Two methods were used to estimate the amount of cell surface lectins which, in this species, are collectively called purpurin. First, the amount elutable from the surface with lactose, a sugar that interacts with its active site, was quantified with a radioimmunoassay. Only about 2% of the total purpurin could be eluted in this manner. In contrast, if the cells were disrupted by freezing and thawing, more than 99% of the total cellular purpurin could be rendered soluble by extraction with lactose, and essentially none remained in a particulate form. Since all the cellular lectin can be extracted with lactose but only 2% can be eluted from the cell surface with this sugar, only this small fraction is inferred to be localized on the cell surface [17]. This conclusion was supported by quantitative binding studies with ^{125}I-Fab prepared from immunoglobulin raised against purpurin [17]. In some experiments the number of Fab fragments bound to the cell surface was actually lower than the number of molecules that could be eluted from the cell surface, but in other experiments these two closely approximated each other. When taken together, the results show that there are about 1×10^5 purpurin molecules on the surface of a slime mold cell. This is only 2% of the 5×10^6 purpurin molecules in each aggregating cell [17].

Although this limited externalization of slime mold lectins seemed puzzling, subsequent studies suggested that more marked externalization could be achieved in specific circumstances. The first evidence for this came from studies of the binding of intact ^{125}I-IgG raised against purpurin. When measurements were made with this reagent, the number of specific ^{125}I-IgG molecules bound to the cell surface of aggregating D purpureum cells was in the range of 1×10^6 [17] about ten times more than with the univalent Fab prepared from this IgG. A number of controls were done to rule out the possibility that this might be an artifact. They showed that the IgG binding was specific, and that the IgG was not releasing lectin by breaking the cells [17].

The results of the IgG binding studies suggested that cross-linking of available endogenous cell surface lectin with antibody directed against it could be a stimulus to the externalization of more intracellular lectin held in a reserve. Presumably divalent IgG directed against cell surface lectin could produce this effect, whereas the univalent antibody fragments could not. Were this the case, other divalent or multivalent reagents that bound to the cell surface lectin and cross-linked it should have a similar effect, whereas univalent ligands like lactose should not. To test this hypothesis we used several polyvalent saccharide-containing molecules that were complementary to the lectin [18]. We first tried lacto-*N*-neotetraose-BSA, a neoglycoprotein, made by incorporation [19] of 31 moles of saccharide per mole of bovine serum albumin. As expected, this material bound well to purpurin.

The neoglycoprotein elicited the externalization of additional purpurin molecules, whereas underivatized bovine serum albumin had no effect (Table I). Likewise, asialo-bovine submaxillary mucin and a bacterial polysaccharide, which are also polyvalent reagents that can cross-link surface purpurin, elicited the appearance of more surface lectin [18]. In the latter experiments, the amount externalized was measured by ^{125}I-anti-lectin Fab binding to the cell surface.

Since two types of reagents that cross-link endogenous cell surface lectins caused the appearance of additional lectin on the surface, we considered the possibility that other reagents that cross-link surface molecules might have a similar effect. To evaluate this we studied the effect of concanavalin A, which is known to bind well to slime mold cell surfaces, and compared it with a succinylated concanavalin A derivative, which would be expected to have less effect in cross-linking cell surface components. We found that Con A could, indeed, promote externalization of additional cell surface lectin up to a maximum of about twice normal [18]. In contrast, succinyl Con A had no effect. However, addition of anti-Con A that cross-linked the succinyl Con A bound to the surface produced an effect similar to that of native Con A. These studies showed that externalization of cell surface lectin may be produced by cross-linking cell surface molecules besides endogenous lectins. Whether or not the effect of Con A requires interaction with molecules that are also receptors for the endogenous lectins is not known.

We also considered the possibility that an excess of added purpurin could elicit externalization of endogenous purpurin. Added purpurin could presumably act by cross-linking available cell surface receptors. To investigate this we differentiated slime mold cells in the presence of ^{14}C-acetate, which was incorporated into the endogenous lectin that was synthesized [18]. We then reacted these labeled cells with an excess of unlabeled purified purpurin, and measured the amount of radioactive purpurin both on the surface and in the medium. We found that reaction of the cells with unlabeled purpurin caused externalization of about five times as much labeled purpurin as was found in the absence of this treatment (Table II). Some of the externalized labeled lectin remained associated with the cell surface and was detected by elution with lactose. A substantial amount was also found in the medium (Table II), presumably by exchanging with the unlabeled soluble pur-

TABLE I. Externalization of Purpurin With Exposure of D purpureum to Lacto-*N*-Neotetraose-BSA

	External purpurin molecules per cell ($\times 10^{-4}$)		
Treatment	Cell surface	Medium	Total external molecules
BSA	7	2	9
Lacto-*N*-neotetraose-BSA	12	6	18

D purpureum cells, dissociated at the aggregating stage, were shaken for 15 min with 0.05 mg/ml of either bovine serum albumin (BSA) or this protein derivatized with 31 moles of lacto-*N*-neotetraose per mole. Cells were separated by centrifugation, and the amount of purpurin in the medium was determined by radioimmunoassay [17]. Cell surface purpurin was eluted with 20 mM lactose as described previously [17] and measured by radioimmunoassay. For details see [18].

purin. These results suggest that cross-linking of available unoccupied cell surface receptors on the surface of slime mold cells by exogenous lectin elicits the externalization of additional lectin. This reaction could be functionally significant. Contact between slime mold cells might foster mutual cross-linking of unoccupied receptors on each cell and unoccupied binding sites of lectin on the surface of the other cells. This could lead to the externalization of more lectin, augmenting this or other cellular interactions.

CONCLUSION

Although far from complete, the evidence reviewed here suggests that lectins, both in chicken tissues and in cellular slime molds, may be held intracellularly in a reserve form as a prelude to their externalization. The cases considered in detail are each different. In chicken intestine, CLL-II is localized in the secretory vesicles of goblet cells, is found on the surface of the intestinal mucosa, and is externalized in response to administration of an agent known to stimulate mucin secretion. In this instance externalization appears to occur by fusion of vesicles with the plasma membrane, a classical secretory mechanism. In the case of the externalization of CLL-I in developing muscle, a somewhat different mechanism is apparently used. As with CLL-II in goblet cells, we presume that lectin passes through an intracellular membrane in the course of its synthesis and processing. However, in muscle, externalization apparently occurs by migration in intracellular tubules, which become continuous with the cell exterior, whereas in the intestine, externalization is achieved only after fusion of secretory vesicles with the plasma membrane.

TABLE II. Externalization of Endogenously Labeled ^{14}C Purpurin From D purpureum With Exposure to Exogenous Purpurin

	Percent of total cellular purpurin		
Treatment	Medium	Surface	Total external
None	0	1.0	1.0
Purpurin	2.1	2.9	5.0

D purpureum cells were differentiated in the presence of ^{14}C-acetate, dissociated at the aggregating stage and shaken with 1 mg/ml of either BSA or purified purpurin [16] for 30 min. Cells were separated by centrifugation, and the medium was saved. Cell surface purpurin was eluted with 20 mM lactose [17]. The medium, the eluate, and aliquots of total D purpureum cell extracts (prepared by freezing cells in 150 mM lactose) were each mixed with 5 ml of Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, New Jersey) and dialyzed overnight to remove lactose and to facilitate purpurin binding to the beads. The dialyzed Sepharose samples were added to a small Sepharose column, and the purpurin bound to each was purified by specific elution [16]. The amount of endogenous purpurin purified from each sample was determined by counting in a liquid scintillation counter. Since total cellular purpurin is extractable by freezing in 150 mM lactose, the total radioactivity in purpurin per cell could be determined and provided the basis for expression of the data on externalized labeled purpurin as percent of total cellular purpurin. For details see [18].

The mechanisms of externalization in cellular slime molds have been studied by biochemical and immunological rather than histochemical methods. Immunohistochemical studies of this lectin in aggregating slime mold cells have thus far shown no obvious localization of lectin in secretory structures, at the level of resolution of the light microscope. However, more definitive localization studies with the electron microscope are required before we can infer the mechanism of externalization in response to surface cross-linking reactions.

Awareness that lectins may be externalized should help in the analysis of their various functions. The finding that lectins are intracellular might suggest an intracellular function. This inference could well be incorrect, however, just as the finding that neurotransmitters are intracellular might lead to the erroneous conclusion that they function within a cell rather than in intercellular interactions. In the present study we have analyzed three cases in which intracellular lectins are externalized. The differences in these externalization reactions suggest that there may be a variety of controls regulating this process. These, in turn, would determine the timing, localization, and degree of potential interaction of these carbohydrate-binding proteins with the complementary cell surface and extracellular heterosaccharides with which they are presumed to associate functionally.

ACKNOWLEDGMENTS

This research was supported by grants from the U.S. Public Health Service and the McKnight Foundation and by the Veterans Administration Medical Center. Eric Beyer is a doctoral candidate in the Physiology-Pharmacology program and is supported by a Medical Science Trainee Award (GMO7198). Douglas Cooper is a doctoral candidate in the Department of Biology and is supported by a research training grant from the NIMH (MH16109-01). We thank Dr. Victor Ginsburg for the gift of lacto-*N*-neotetraose conjugated to bovine serum albumin.

REFERENCES

1. Barondes SH: Ann Rev Biochem 50:207, 1981.
2. Neufeld EF, Ashwell G: In Lennarz W (ed): "The Biochemistry of Glycoproteins and Proteoglycans." New York: Plenum Press, 1980, pp 241–266.
3. Nowak TP, Kobiler D, Roel L, Barondes SH: J Biol Chem 252:6026, 1977.
4. Den H, Malinzak DA: J Biol Chem 252:5444, 1977.
5. Beyer EC, Zweig SE, Barondes SH: J Biol Chem 255:4236, 1980.
6. Ceri H, Kobiler D, Barondes SH: J Biol Chem 256:390, 1981.
7. Beyer EC, Barondes SH: J Cell Biol (in press).
8. Mir-Lechaire F, Barondes SH: Nature 272:256, 1978.
9. Roberson MM, Ceri H, Shadle PJ, Barondes SH: J Supramol Struct Cell Biochem 15:395, 1981.
10. Beyer EC, Barondes SH: J Cell Biol (in press).
11. Barondes SH, Haywood-Reid P: J Cell Biol (in press).
12. Beyer EC, Tokuyasu K, Barondes SH: J Cell Biol 82:565, 1979.
13. Beyer EC, Barondes SH: J Supramol Struct 13:219, 1980.
14. Ceri H, Shadle PJ, Kobiler D, Barondes SH: J Supramol Struct 11:61, 1979.
15. Rosen SD, Kafka JA, Simpson DL, Barondes SH: Proc Natl Acad Sci USA 70:2554, 1973.
16. Cooper DN, Barondes SH: J Biol Chem 256:5046, 1981.
17. Springer WR, Haywood PL, Barondes SH: J Cell Biol 87:682, 1980.
18. Springer WR, Barondes SH: (Submitted for publication).
19. Zopf DA, Tsai CM, Ginsburg V: Arch Biochem Biophys 185:561, 1978.