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Purification and Properties of Lung Lectin

RAT LUNG AND HUMAN LUNG β-GALACTOSIDE-BINDING PROTEINS

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Lung is one of the organs of the rat with a particular abundance of haemagglutinating activity that is inhibited by β-galactosides. This lectin activity can be attributed to a single protein that has been purified from rat lung; a similar protein has been purified from human lung. The molecular weights and subunit structures were estimated from gel filtration and sodium dodecyl sulphate/polyacrylamide-gel electrophoresis; the human lung lectin appeared to be composed of two identical subunits, mol.wt. 14500, whereas rat lung lectin was observed as both a dimer and a tetramer of one subunit type, mol.wt. 13500. Both lectins bind to disaccharides or oligosaccharides with terminal β-linked galactose residues. The carbohydrate moiety may be free [lactose or D-galactopyranosyl-β-(1 → 4)-thiogalactopyranoside], protein-bound (asialofetuin) or lipid-bound (cerebrosides). The molecular properties of the β-galactoside-binding proteins of rat lung and human lung are closely similar to those of embryonic chick muscle lectin [Nowak, Kobiler, Roel & Barondes (1977) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1383–1387] and calf heart lectin [De Waard, Hickman & Kornfeld (1976) *J. Biol. Chem.* **251**, 7581–7587].

Several vertebrate saccharide-binding proteins have been described. These proteins agglutinate erythrocytes in a saccharide-sensitive reaction; they have therefore been called lectins. Two classes of vetebrate lectins can be distinguished. The first, chronologically, comprises a group of high-molecular-weight, Ca^{2+}-dependent, integral membrane proteins that bind glycoproteins, namely the mammalian hepatic lectin specific for galactose (Hudgin *et al.*, 1974; Kawasaki & Ashwell, 1976) and another specific for fucose α-(1→3)-*N*-acetylglucosamine linkages (Prieels *et al.*, 1978), the avian hepatic lectin specific for *N*-acetylglucosamine (Kawasaki & Ashwell, 1977), and an alveolar macrophage receptor specific for glucose and mannose (Stahl *et al.*, 1978). The hepatic lectins are thought to function in the clearance of asialoglycoproteins from serum.

The second class of vertebrate lectin comprises a group of low-molecular-weight β-galactoside-binding proteins specific for terminal galactose β-(1→4)-*N*-acetylglucosamine and galactose β-(1→4)-galactose moieties; these lectins can be extracted and inhibited by lactose. They were first reported in the electric organ of the electric eel (*Electrophorus Electricus*), various rat tissues, chick myoblasts and mouse cell lines (Teichberg *et al.*, 1975). The purified lectin from electric-organ tissue (Teichberg *et al.*, 1975) is similar in size (mol.wt. 33000) and saccharide specificity to the lectins later purified from embryonic chick myoblasts (Nowak *et al.*, 1977; Den & Malinzak, 1977). Various calf tissues, including heart and lung (De Waard *et al.*, 1976; Briles *et al.*, 1979), rat lung (Powell, 1979) and neural tissue (Eisenbarth *et al.*, 1978). These β-galactoside-binding lectins are developmentally regulated in embryonic chick muscle (Nowak *et al.*, 1977; Kobiler & Barondes, 1977) and rat lung (Powell, 1979). There has been speculation that these β-galactoside-binding proteins may be involved in synaptogenesis (Teichberg, 1979) and the specific adhesion and fusion of myoblasts (Gartner & Podleski, 1975). It has been suggested that these lectins are ubiquitously distributed among vertebrates (Teichberg *et al.*, 1975), but their physiological role remains to be deciphered. The present paper describes the isolation of rat lung lectin and human lung lectin and compares some of their properties.

Abbreviation used: thiodigalactoside, D-galactopyranosyl β-(1→4)-thiogalactopyranoside.

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Experimental

p-Aminophenyl β-D-lactoside was obtained from Vega Biochemicals, Tucson, AR, U.S.A., and *N*-palmitoyl DL-dihydrolactocerebroside from Miles Laboratories, Elkhart, IN, U.S.A. Other saccharides, trypsin, ovalbumin, lysozyme, cytochrome *c*, bovine serum albumin and fetuin were obtained from Sigma, St. Louis, MO, U.S.A. Carbonic anhydrase was a gift from Dr. P. L. Whitney of this Department. Bio-Gel P10, P100 and reagents for gel electrophoresis were obtained from Bio-Rad Laboratories. Richmond, CA, U.S.A. Activated-thiol–Sepharose 4B was purchased from Pharmacia, Piscataway, NJ, U.S.A. ^{125}I-labelled Bolton–Hunter (1973) reagent was purchased from New England Nuclear, North Billerica, MA, U.S.A. Outdated human plasma was purchased from the John Elliot Blood Bank, Miami, FL, U.S.A. Ultrafiltration of solutions was performed in an Amicon apparatus, model 10PA, with UM10 or YM10 membranes (Amicon, Lexington, MA, U.S.A.). Whatman GF/C and GF/F filters were obtained from a local supplier. Other reagents were of the best commercially available grade. Male Long-Evans hooded rats (75–100g) were obtained from Charles River Farm, Wilmington, MA, U.S.A. Sections of normal human lung were obtained from lung surgery, with the informed consent of patients.

Lectin Assay by haemagglutination

Agglutination assays were performed in Cooke microtitre plates by using trypsin-treated rabbit erythrocytes, fixed and unfixed (Simpson *et al.*, 1977). Agglutination was considered positive only if it could be inhibited by lactose (2.5mM) and thiodigalactoside (0.5mM). The titre was read as the last serial dilution to effect agglutination; 1 unit of lectin activity is defined as the ability to agglutinate in the above assay at 1000-fold dilution, or, 1 unit equals 1000 titre units.

Lectin assay by asialo-orosomucoid binding

Orosomucoid was purified from human serum as previously described (Whitehead & Sammons, 1966). Terminal sialic acid residues were removed by incubating the protein at 80°C at pH2 for 1h. CNBr cleavage of asialo-orosomucoid (Steers *et al.*, 1965) yielded only one glycosylated fragment (Schmid *et al.*, 1973). This fragment was labelled with ^{125}I with the Bolton–Hunter reagent and separated from the reaction mixture by gel filtration on Bio-Gel P100 (0.9cm × 30cm); the specific radioactivity of the glycosylated fragment was 0.1mCi/mg. The use of the glycosylated CNBr fragment of asialo-orosomucoid avoids the problem of non-specific binding observed with intact asialo-orosomucoid. [^{125}I]Asialo-orosomucoid could be used to estimate pure lectin, but not the lectin in crude solutions.

Lectin (0–4μg) was incubated with 25μg of labelled glycosylated asialo-orosomucoid fragment in a total volume of 0.2ml at 22–25°C for 20min. All assay components were in buffer A (0.14M-NaCl containing 20mM-sodium phosphate, pH7.5, and 10mM-2-mercaptoethanol). After 30min, 40% (w/v) poly(ethylene glycol)-6000 in 10mM-sodium phosphate, pH7.5 (0.18ml) was added, the samples mixed well and chilled in ice for 30min. The samples were filtered under suction on GF/C filters, reaction vials rinsed with 0.6ml of 20% poly(ethylene glycol) in 10mM sodium phosphate, pH7.5 [20% poly(ethylene glycol)]. The filter was washed with 20% poly(ethylene glycol) (2ml) followed by 60% (v/v) acetone in water (5ml) and counted for radioactivity in a Packard Autogamma counter. Control assays contained 5mM-thiodigalactoside. This assay was linear for 0–4μg of lectin. Pure rat lung lectin bound 0.8mg of orosomucoid fragment/mg of protein and pure human lung lectin bound 0.86mg of orosomucoid fragment/mg of protein under these conditions.

Protein assays

Protein was determined by using the dye-binding method (Bradford, 1976).

Disc gel electrophoresis

Polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate was used to determine subunit molecular weights (Weber & Osborn, 1969). The following marker proteins were used: cytochrome *c* (mol.wt. 12400), lysozyme (14300), carbonic anhydrase (28000) and ovalbumin (43000). Bromophenol Blue was used as the tracking dye and gels were stained with Coomassie Brilliant Blue.

Amino acid analysis

Samples were hydrolysed in 4M-methanesulphonic acid at 100°C for 20h and 70h and analysed on a Jeol 5AH amino acid analyser. Serine and threonine values were extrapolated to zero time and the valine value taken from the 70h hydrolysis. Samples for cysteine determination were oxidized with performic acid (Moore, 1963) and hydrolysed in 6M-HCl at 100°C for 18h.

Preparation of affinity-chromatography matrices

Bio-Gel P100 (100–200 mesh) was converted into a hydrazide derivative with 6M-hydrazine at 22°C (Inman, 1974). The acyl azide produced by diazotization (Inman, 1974) was allowed to react separately with *p*-aminophenyl β-D-lactoside and asialo-fetuin in 0.1M-borate buffer, pH8.6.

Erythrocyte glycoprotein

Glycoprotein from rat erythrocytes was prepared by the method of Emerson & Kornfeld (1976) and sialic acid was removed by acid hydrolysis (pH 2, 1 h, 80°C).

Cerebroside micelles

Phospholipid/glycolipid micelles were prepared essentially as described by Curatolo *et al.* (1979); 10 mol% *N*-palmitoyl DL-dihydrolactocerebroside vesicles were prepared in buffer A.

Purification of lectin

(i) From rats: rats were given an intraperitoneal injection of sodium pentobarbital (30 mg/kg) and killed by exsanguination from the abdominal aorta; the lungs were removed, trimmed and frozen in liquid N_2; and (ii) from humans: fresh human lung, washed free of blood, was sliced into cubes and frozen in liquid nitrogen. Frozen tissue was disrupted by using a Polytron (Brinkman Instruments, Westbury, NY, U.S.A.) with a ratio of 6 ml of homogenizing medium to 1 g of lung. The homogenizing medium was 0.25 M-sucrose containing 10 mM-lactose, 20 mM-sodium phosphate, pH 7.5, and 10 mM-2-mercaptoethanol. The homogenate was centrifuged at 5000 *g* for 30 min at 4°C, the pellet discarded, and the supernatant was centrifuged at 100000 *g* for 1 h at 4°C and the pellet again discarded. The lectin purification proceeded by fractionation of the supernatant solution. In different tissues lectin activity was determined in this solution after dialysis against buffer A (100000 *g* centrifugation) with $(NH_4)_2SO_4$; the fraction precipitating between 30 and 55% saturation was suspended in 0.14 M-NaCl containing 20 mM-sodium phosphate, pH 7.5, and 10 mM-mercaptoethanol and dialysed against 200 vol. of the same buffer (buffer A). Insoluble protein was removed by centrifugation and the active fraction applied to a column of asialo-fetuin–Bio-Gel P100. The column (1.6 cm × 12 cm) was equilibrated with buffer A at a flow rate of 5 ml/h. When the lectin was loaded, the column was washed with buffer A until the A_{280} was zero. The column was then developed with buffer A containing 0.1 M-lactose. A 'sharp peak' of protein was eluted that after dialysis against buffer A (to remove lactose) was shown to contain the lectin. Lectin was concentrated to 1 ml by ultrafiltration and high-molecular-weight contaminants were removed by gel filtration on a column of Bio-Gel P100 (0.9 cm × 40 cm) equilibrated with buffer A at 2.5 ml/h. Contaminants eluted at or near the void volume of the column, whereas the lectin was retained. The same column of Bio-Gel P100 was calibrated with Blue Dextran, UMP and proteins of known molecular weight [bovine serum albumin (68000), ovalbumin, carbonic anhydrase and cytochrome *c*].

Alternatively, affinity chromatography of lectin could be achieved on a column of *p*-aminophenyl β-D-galactoside linked to Bio-Gel P100 (1.2 cm × 5 cm). This column was equilibrated and developed with buffer A at 2 ml/h. Thiodigalactoside (5 mM) was added to the buffer to elute the lectin; lectin activity was recovered after dialysis against buffer A.

Lectin stability

The pure lectins were stable to storage at 4°C in the presence of 1 mM-lactose in buffer A for 2–3 months. Impure samples were stable to freezing at −20°C for months; pure lectin was not stable at −20°C.

Results and discussion

A survey of different issues of the rat showed a particular abundance of lectin in heart (1.3 ± 0.4 units/mg of protein), lung (1.6 ± 0.4 units/mg of protein) and uterus (female rats: 2.8 ± 1 units/mg of protein). Other tissues tested, containing less than 0.2 units/mg of protein, included kidney, spleen, oesophagus, gut, bladder, thymus and soleus muscle. It remains possible that the efficiency of protein extraction and the presence of endogenous inhibitors may vary from tissue to tissue and contribute in small part to the apparent specific localization of lectin. Within the lung itself, lectin cannot be extracted by airway lavage, in the presence of lactose, which would imply that the lectin was not present in macrophages or on the surface of airway epithelial cells. Neither was the lectin released by vascular perfusion in the presence of lactose, which suggested that lectin was not present on the endothelial-cell surface.

The presence of lactose in the homogenizing buffer markedly facilitates the extraction of lectin (Table 1). For instance, the buffer used for the extraction of angiotensin-converting enzyme from rat lung, 0.02 M-potassium phosphate, pH 8.3 (Lanzillo & Fanburg, 1974) solubilized only a small portion of the lectin activity. The optimal lactose concentration (10 mM) in 0.25 M-sucrose permitted efficient extraction of lectin, about 75% with a single homogenization. This would suggest that the lectin is held *in situ* by at least one of its galactose-binding sites.

The β-galactoside-binding protein (or lectin) has been purified to homogeneity from both human lung and rat lung. A summary of the purification procedure is given in Table 2, illustrating the alternative affinity-chromatography steps. The yield of pure lectin was about 0.05% of the soluble protein. The criterion of purity was that each lectin migrated as a single band on polyacrylamide-gel

electrophoresis [10% (w/v) polyacrylamide gels] at both pH 8.3 and pH 9.2. Electrophoresis in the presence of sodium dodecyl sulphate also showed a single band for each lectin (Fig. 1). The human-lung-lectin subunit appeared slightly larger (mol.wt. 14500) than the rat-lung-lectin subunit (mol.wt. 13500).

These subunit molecular weights, 14500 for human lung lectin and 13500 for rat lung lectin, are similar to those reported for embryonic chick muscle lectin [15000 (Nowak *et al.*, 1977)] and for calf heart lectin [12000 (Briles *et al.*, 1979)]. Both the embryonic chick muscle lectin and the calf lectins have dimeric native forms (mol.wts. 30000 and 24000 respectively).

Table 1. *Lectin extraction from rat lung*

For each experiment, 1 g of rat lung was disrupted in 6 ml of homogenizing medium and the results taken from 100000*g* supernatant. The final two lines show the re-extraction of the combined 5000*g* and 10000*g* pellets. All the media contained 10 mM-2-mercaptoethanol and 20 mM-sodium phosphate, pH 7.5.

Homogenizing medium	Specific activity of lectin (units/mg)	Total units
0.02 M-Potassium phosphate, pH 8.3	0	0
0.14 M-NaCl	0.4 ± 0.1	5.6 ± 1
0.14 M-NaCl + 10 mM-lactose	0.9 ± 0.3	13.2 ± 2.2
0.5 M-NaCl	0.4 ± 0.1	5.3 ± 1
0.5 M-NaCl + 10 mM-lactose	0.7 ± 0.2	9.2 ± 1.7
0.25 M-Sucrose	0.5 ± 0.2	8.8 ± 2
0.25 M-Sucrose + 10 mM-lactose	1.6 ± 0.4	25.6 ± 2
0.25 M-Sucrose + 10 mM-lactose (re-extraction of pellet from above)	1.3 ± 0.4	6.4 ± 2.2
0.25 M-Sucrose + 10 mM-lactose (re-extraction of pellet from above)	0.2 ± 0.1	0.8 ± 0.4

Gel filtration on a calibrated column of Bio-Gel P100 permitted the evaluation of the molecular weights of native human lung lectin and rat lung

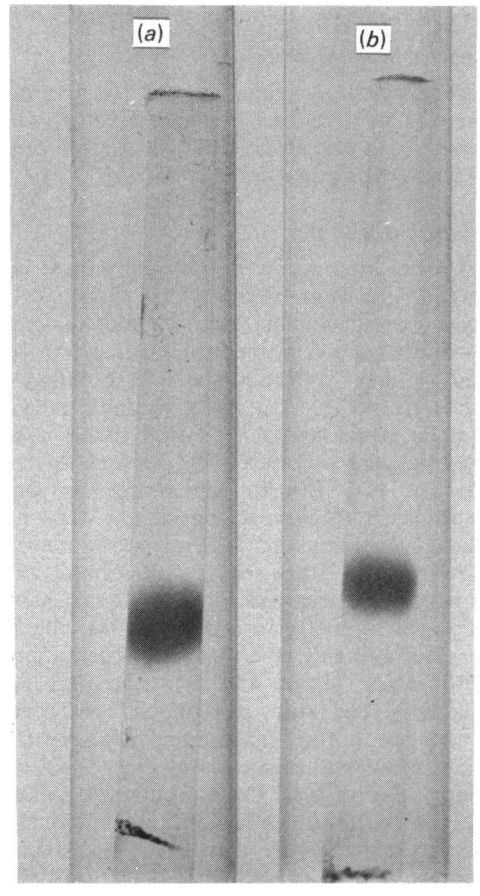

Fig. 1. *Gel electrophoresis in the presence of sodium dodecyl sulphate of purified (a) rat lung lectin and (b) human lung lectin*

The marks at the bottom of the gels indicate the position of the tracking dye.

Table 2. *Purification of lectin from rat lung and human lung*

The specific activity of pure lectin varied slightly from preparation to preparation, 450–540 units/mg was recorded for human lung and 480–600 units/mg for rat lung.

	Total protein (mg)		Total lectin (μg)		Lectin specific activity (units/mg)		Yield (%)	
	Rat	Human	Rat	Human	Rat	Human	Rat	Human
5000*g* supernatant	410	370	487	150	1.2	0.41		
100000*g* supernatant	330	281	458	136	1.4	0.48	93	91
30–50% $(NH_4)_2SO_4$	85	55.2	442	115	5.3	2.08	92	77
Asialo-fetuin P100 chromatography	1.8		295		163		61	
p-Aminophenyl β-D-lactoside–Bio-Gel P100 chromatography		0.4		88		220		59
Gel filtration (Bio-Gel P100)	0.39	0.152	210	75	535	493	43	50

lectin as 28000±3000 and 26000±3000 respectively. Both these lectins appear to have dimeric structures in the presence of 2-mercaptoethanol (10mM). The presence of lactose (10mM) had no effect on the elution profiles. However, in the presence of lower concentrations of reducing agent [dithiothreitol (0.2mM) or 2-mercaptoethanol (2mM)] the rat lung lectin appeared to be a tetramer with a molecular weight estimated as 60000±6000; human lung lectin still appeared dimeric under these conditions [mol.wt. 28000]. The specific activity of the dimeric and tetrameric molecular forms of rat lung lectin were very similar. The maintenance of lectin activity, both from rat lung and human lung, is dependent on the presence of reducing thiol groups (2-mercaptoethanol or dithiothreitol). This is another property in common with calf lectins (Briles *et al.*, 1979) and embryonic chick muscle lectins (Nowak *et al.*, 1977; Den & Malinzak, 1977), and is indicative of a sensitive cysteine residue that is conserved in all of these β-galactoside-binding proteins. Consistent with this idea, both rat lung lectin and human lung lectin bind to activated-thiol–Sepharose 4B and can be specifically eluted from the matrix with L-cysteine. The amino acid compositions of rat lung lectin and human lung lectin suggests that each has but a single cysteine residue or subunit. The compositions of these two lectins (shown in Table 3) are very similar; the human lung lectin may be more heavily glycosylated than the rat lung lectin, which could perhaps account for the molecular-weight differences. For each lectin the amino acid composition was constant from preparation to preparation.

The saccharide specificity of the lung lectins is closely similar to that of calf heart lectin (De Waard *et al.*, 1976) and embryonic chick muscle lectin (Nowak *et al.*, 1977). Saccharide specificity was assessed both by inhibition of haemagglutination and by inhibition of the orosomucoid-fragment-binding assay. The results are given in Table 4. Galactose and other monosaccharides were ineffective as lectin inhibitors. Terminal sialic acid residues impaired hapten binding; fetuin was less than 20% as efficient as asialo-fetuin in inhibiting lectin-induced haemagglutination. Thiodigalactoside was a much more potent inhibitor than lactose. The lung lectins were not inhibited by galactose-containing polymers of plant origin with predominantly

Table 3. *Amino acid composition of rat lung lectin and human lung lectin*

The amino sugars comprise glucosamine and galactosamine. Cysteine was determined as cysteic acid.

	Composition (residues/molecule of subunit)	
	Rat lung	Human lung
Cys	1	1
Asx	11	9
Thr	6	6
Ser	17	19
Glx	20	18
Pro	5	5
Gly	15	19
Ala	9	10
Val	5	6
Met	1	1
Ile	3	4
Leu	6	6
Tyr	3	2
Phe	2	2
His	4	4
Trp	1	1
Lys	4	4
Arg	3	3
Amino sugars	2	3
Total residues	116	120

Table 4. *Inhibition characteristics of rat lung and human lung lectins*

I is the concentration of hapten required to inhibit the agglutination effected by 1 unit of lectin (2μg). *K* is the concentration of hapten required to inhibit the binding (by 50%) of 1 unit of lectin (2μg) to asialo-orosomucoid fragment. Abbreviation used: n.d., not determined.

	I (mM)		*K* (mM)	
Hapten	Rat lung	Human lung	Rat lung	Human lung
Galactose	>25	>25	>25	>25
N-Acetylgalactosamine	>25	>25	n.d.	n.d.
N-Acetylglucosamine	>25	>25	>25	>25
Lactose	1.5	0.5	2.6	1.6
Thiodigalactoside	0.25	0.09	0.42	0.28
Asialo-fetuin	0.54	0.32	n.d.	n.d.
Rat erythrocyte asialo-glycoprotein (reducing termini)	0.12	0.09	0.18	0.10

α-linked galactose residues, e.g. arabinogalactans and galactomannans. Rat erythrocyte asialoglycoprotein was a potent inhibitor of rat lung lectin; it was an even more potent inhibitor of the human lung lectin (see Table 4). It remains unlikely that erythrocyte glycoprotein is a substrate for the lectin *in vivo* and that the haemagglutination has any physiological relevance. Lung lectin seems well shielded from blood under normal conditions, since it was not released by vascular perfusion with lactose. It remains possible that lectin plays a role in pulmonary haemorrhage. The binding specificity for both rat and human lung lectin is for terminal β-linked galactose residues of oligosaccharides and glycoproteins. The inhibition constants for lactose and thiodigalactoside reported here for lung lectins are of the same order of magnitude as those reported for calf heart lectin (De Waard *et al.*, 1976) and embryonic chick lectin (Nowak *et al.*, 1977).

The ability of castor-bean (*Ricinus communis*) lectin to aggregate glycolipid-containing vesicles has been reported (Curatolo *et al.*, 1978); this aggregation was monitored by the increasing A_{450}. I used the same methodology to show that rat lung lectin and human lung lectin (pure lectin at a concentration of 10μg/ml) caused the aggregation of cerebroside micelles (10mol% *N*-palmitoyl DL-dihydrolactocerebroside). The lung-lectin-induced aggregation of the cerebroside micelles could be specifically inhibited by the presence of thiodigalactoside (1mM). The concentration of thiodigalactoside required to inhibit cerebroside-micelle formation is double that required to inhibit haemagglutination; neither phenomenon is sufficiently well understood to make comment on this. The aggregation of cerebroside micelles was not explored further, but these preliminary experiments suffice to demonstrate that glycolipids are a possible target for lectin binding *in vivo*; the pertinent sugar moieties, terminal β-linked galactose, are found in globosides (Yamakawa & Nagai, 1978). The present data do not allow discrimination as to the lectins' substrate(s) *in vivo*.

These lung lectins have but one observable function, to bind β-galactosides. Two other classes of protein possess this same characteristic: β-galactosidase (glycosidase) and sialyltransferase (glycosyltransferase). The human lung lectin and rat lung lectin were devoid of these activities.

The physiological role of these lectins remain unknown. We assume that Nature has been magnanimous and ascribed an important function to these lectins. Their molecular properties suggest that they are excellent candidates for the mediation of a recognition process, perhaps a cell-to-cell recognition process through surface glycoproteins, or perhaps they function as receptors for a regulatory glycopeptide or glycoprotein. Further, it appears that the role assumed by the lectins is of critical relevance at a specific state of development (Nowak *et al.*, 1977; Kobiler & Barondes, 1977; Simpson *et al.*, 1977); this is also true for the lung, where lectin activity reaches a peak at the height of alveolarization (Powell, 1979; Powell & Whitney, 1980).

Attempts have been made to localize lung lectin with immunofluorescence techniques. Lectin appears to be present on the surface of, and within, myoblasts in embryonic chick pectoral muscle (Nowak *et al.*, 1977) and in neuronal-cell bodies and neuronal-cell surfaces in embryonic chick brain (Gremo *et al.*, 1978). Immunofluorescence studies with cultured cell lines have suggested a predominantly intracellular localization (Briles *et al.*, 1979). This might suggest that the lectin functions to bind and internalize a regulatory ligand in a manner similar to the hepatic binding protein (Tanabe *et al.*, 1979). Studies are required to evaluate the function and location of β-galactoside-binding protein in postnatal lung development.

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