VERTEBRATE LECTINS

Comparison of Properties of β-Galactoside-Binding Lectins from Tissues of Calf and Chicken

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ABSTRACT

β-Galactoside-binding lectins were isolated from various calf tissues and from chicken hearts by affinity chromatography on asialofetuin-Sepharose, and were compared with respect to biochemical characteristics, binding properties, antigenic cross-reactivity, and cellular localization. The lectins are all thiol group-requiring, divalent cation-independent dimers, of apparent monomer mol wt 12,000 (calf lectins) or 13,000 (chicken lectin), and acidic pI. The calf lectins appear essentially identical by dodecyl sulfate-polyacrylamide gel electrophoresis, isoelectric focusing, amino acid composition, and radioimmunoassay, while the chicken lectin is distinctly different by these criteria. However, all of the lectins competed for the same binding sites on rabbit erythrocytes, and could be inhibited by the same saccharide haptens (notably lactose and thiodigalactoside). Immunofluorescence studies on several cultured cell lines revealed that the bovine and chicken lectins had primarily an intracellular cytoplasmic localization. The β-galactoside-binding lectins of vertebrates appear to be species-specific rather than tissue-specific.

KEY WORDS lectins · vertebrate lectins · β-galactoside-binding lectins · affinity chromatography

Although lectins from various plant and invertebrate sources have been known for many years (for a comprehensive list, see reference 23), their presence in vertebrate tissues has been investigated only recently. Ashwell and Morell (3) have described a hepatic binding protein which has been implicated in the clearance of glycoproteins from plasma; in mammals, this binding protein is a β-galactoside-specific, integral membrane protein of large molecular weight, which can be solubilized by detergents but not by hapten saccharides and which requires divalent cations for binding activity. Subsequently, hepatic and reticuloendothelial cell-binding proteins which recognize mannose, *N*-acetylglucosamine, and fucose have been detected (1, 4, 6, 8, 18, 20, 29, 31, 32). A "lectin" from platelet plasma membranes has been described by Gartner et al. (16) which is inhibitable by free amino sugars and amino acids; the large external transformation-sensitive (LETS) protein or fibronectin also agglutinates erythrocytes and is inhibitable by amines (35). In addition to these membrane-bound lectins, several

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soluble lectins have been identified in vertebrate tissues. Mir-Lechaire and Barondes (25) have reported a lectin from chick embryo muscle which is specific for *N*-acetylgalactosamine. In addition, a group of small molecular weight, β-galactoside-specific lectins has been isolated from such diverse sources as electric eel electric organs (33), chick embryo myoblasts (11, 15, 21, 28) and neural tissue (14), and calf heart and lung (12), and are probably distributed ubiquitously among vertebrates (33). These lectins share a specific requirement for thiol-reducing groups, and do not require divalent cations for agglutinating activity. Although some investigators have speculated that these lectins might play a role in the specific adhesion and fusion of myoblasts during embryonic development (11, 15, 21, 28), the physiologic role of these lectins in vivo has yet to be established.

In the present report, we compare the β-galactoside-specific lectins from various calf tissues and from adult chicken hearts. The calf lectins are all identical to each other, as far as we can tell, and similar to, yet clearly distinct from, the chicken lectin. We also present evidence for the intracellular localization of these lectins.

MATERIALS AND METHODS

Isolation of Lectins

Fresh calf organs (liver, spleen, heart, thymus) and chicken hearts were obtained from local slaughterhouses. Soluble and particulate-associated lectins were isolated by affinity chromatography on asialofetuin-Sepharose, essentially as described by de Waard et al. (12), with the following modifications: (*a*) The buffer used throughout, SPB, contained 0.14 M NaCl, 0.02 M KPO_4, pH 7.4, 0.014 M β-mercaptoethanol, and 0.02% NaN_3; additionally, 0.25 mM phenylmethylsulfonylfluoride was present during initial homogenization of the tissues; (*b*) the particulate-associated lectin was obtained in soluble form by extraction with 0.1 M lactose in homogenization buffer at 5°C overnight. In some calf spleen lectin preparations, the splenic pulp was scraped out of the capsule of either fresh or partially thawed spleens, to facilitate homogenization; all of the lectin activity of whole spleens was found to be in the splenic pulp. Lectin binding to the affinity column was monitored by assaying the effluent for agglutinating activity. When the column became overloaded, as indicated by the efflux of agglutinating activity, the excess agglutinin was loaded onto a second column.

Biochemical Characterization of Lectins

SDS gel electrophoresis was performed on a 1-mm-thick, 10-cm-long 10% acrylamide slab gel (2.5% bisacrylamide) with a 0.8-cm-long 4% acrylamide upper gel, in the phosphate buffer system of Weber and Osborn (34) modified by including 14 mM 2-mercaptoethanol in the electrode buffer. The gel was pre-run for 3 h, and the samples (in 30 μl containing 0.07 M NaCl, 0.01 M KPO_4, pH 7.4, 8.5% wt/vol sucrose, 0.002% wt/vol bromphenol blue, 1% wt/vol SDS, 35 mM 2-mercaptoethanol, and 75 mM lactose) were incubated at 100°C for 5 min before loading. Electrophoresis was carried out at 45 mA (1.7 W) for 11 h. The gel was stained with 0.3% Coomassie Brilliant Blue R in 50% vol/vol methanol containing 12% wt/vol TCA, and destained with 7% vol/vol acetic acid in 10% vol/vol methanol.

Gel permeation chromatography was performed on a Bio-Gel P-60 (100- to 200-mesh) column (0.9 × 120 cm; Bio-Rad Laboratories, Richmond, Calif.), with SPB as the eluting buffer; the column was calibrated using myoglobin, cytochrome *c*, and ovalbumin as standards.

Slab isoelectric focusing was performed on an LKB horizontal slab apparatus (LKB Instruments, Inc., Rockville, Md.), using pre-poured pH 3.5–9.5 gels (LKB PAG plate) which were sprayed lightly with 1.5 M 2-mercaptoethanol just before use. The gel was fixed in 15% aqueous TCA for ½ h, rinsed with water, and then stained with 0.09% Coomassie Brilliant Blue R in 8.8% methanol-8% acetic acid, and destained with 10% acetic acid-10% methanol. The pH gradient was determined by measuring the pH of 1-cm segments cut from an edge of the gel before fixation, after elution into a minimal volume of water.

Protein was quantitated either by the method of Lowry et al. (24) on samples which were dialyzed free of 2-mercaptoethanol, or by the method of Bradford (7), using bovine serum albumin (BSA) as the standard, or by determining the optical absorption at 280 nm.

Radioiodination of Lectins

In early studies, the lectins were labeled with ^{125}I, after dialysis to remove the mercaptoethanol, by the chloramine-T method (17), using a 10-s exposure to the chloramine-T. We observed, however, that this technique resulted in an unstable association of the label with the lectin. The degree of instability tended to vary widely from batch to batch, and some occasional preparations were actually stable over long periods of storage time. To circumvent this problem, the ^{125}I-lectins were either repurified by affinity chromatography immediately before use, or the amount of ^{125}I label actually bound to the lectin was determined by precipitation with 10% TCA at the time of the experiment. Alternatively, the lectins could be conveniently and stably radioiodinated with ^{125}I-*N*-succinimidyl-3(4-hydroxyphenyl) propionate as described by Bolton and Hunter (5), and this became our method of choice for most of the experiments described herein.

Lectin Binding to Rabbit Red Cells

Binding assays were performed in 1.5-ml plastic mi-

crocentrifuge tubes (Brinkmann Instruments, Inc., Westbury, N. Y.) which had been presoaked with 20 mg/ml BSA before use. Whole rabbit blood was stored in sterile Alsever's solution at 5°C for up to 1 mo; for assays, cells were washed four times with phosphate-buffered saline just before use.

For determination of binding parameters, the reaction mixtures contained 10^7 washed rabbit red cells plus varying amounts of ^{125}I-labeled lectin in 150 μl of phosphate-buffered saline containing 1 μmol of β-mercaptoethanol and 300 μg of BSA. After incubation at room temperature for 2–3 h, 175 μl of "oil" (Apiezon: *n*-butylphthalate, 1:9; $\rho = 1.03$) was added to each sample, and the tubes were centrifuged at 5°C for 7½ min in an Eppendorf microcentrifuge. The upper aqueous phase plus most of the oil layer were removed by aspiration, and the lower tip of the tube, containing the red cell pellet, was cut off with a hot scalpel and assayed for ^{125}I in a gamma counter. Apparent K_d values and the number of binding sites per cell were determined by the Scatchard plot method (30).

To study the inhibition of binding by saccharide haptens or competing lectins, assay conditions were selected such that the lectin and red cell receptor site concentrations were near their apparent K_d values. Thus, per 150 μl of reaction volume, in phosphate-buffered saline containing 1 μmol of 2-mercaptoethanol and 300 μg of BSA plus varying amounts of the saccharide or competing lectin being studied for inhibitory activity, the reaction mixtures included: (*a*) for chicken heart lectin, 0.08 μg of ^{125}I-lectin (0.1 μCi/μg), plus 2×10^6 washed rabbit red cells; (*b*) for calf spleen particle-associated lectin, 2 μg of ^{125}I-lectin (0.005 μCi/μg), plus 2.5×10^7 washed rabbit red cells. The red cells were added last. Cell-bound radioactivity was determined using the oil centrifugation procedure described above. Lactose and thiodigalactoside were purchased from Sigma Chemical Co. (St. Louis, Mo.); α- and β-methylgalactosides were purchased from Pfanstiehl Laboratories, (Waukegan, Ill.).

Radioimmunoassay

Rabbit antisera to soluble calf heart lectin and to purified chicken heart lectin were prepared as previously described (12).

The initial immunoassay reactions were performed in 250 μl of SPB containing 150 μg of BSA, 30 μmol of lactose, competing agglutinins in varying amounts, ^{125}I-labeled lectin probe (either 0.8 μg of calf heart particle-associated lectin, 0.16 μg of calf spleen particulate lectin, or 0.12 μg of chicken heart lectin), and either an amount of antiserum predetermined to complex 50% of the labeled probe in the absence of competitors or an equivalent volume of normal rabbit serum (control). The ^{125}I probe was always added last. After incubation at 37°C for 2 h, the reaction mixtures were chilled, and 1.5 ml of ice-cold 25% wt/vol polyethylene glycol 6,000 in phosphate-buffered saline was added, to induce precipitation of antibody-antigen complexes as described by Creighton et al. (9). After overnight incubation at 0°C, precipitates were collected by centrifugation (Sorvall SS-34 rotor, DuPont Instruments, Wilmington, Del., 12,000 rpm × 30 min), and the precipitated radioactivity was determined in a Beckman gamma counter (Beckman Instruments, Inc., Fullerton, Calif.). In some experiments, up to 10 μl of additional normal rabbit serum was added to each assay tube as "carrier" to facilitate collection of precipitates.

Immunofluorescence Microscopy

Fluorescent antibody was prepared as follows: 800 μl of fluorescein isothiocyanate-conjugated goat anti-rabbit IgG (Gateway Immunosera Co., Cahokia, Ill.) was mixed with an equal volume of 50% washed packed mouse L-cells in normal goat serum (Gateway Immunosera Co.), incubated at 0°C for 15 min, and then centrifuged at 500 *g* for 5 min at 5°C. The supernate was reserved, and the L-cell pellet was resuspended with 2 ml of phosphate-buffered saline and recentrifuged. The two supernates were pooled, clarified by centrifugation for 15 min at 12,000 *g* at 5°C, and stored in 0.5-ml aliquots at −20°C.

Primary chick embryo fibroblasts were a gift from Dr. S. Schlesinger (Washington University), and were used at the second passage; primary bovine embryonic and postnatal kidney cells were purchased from Flow Laboratories (Rockville, Md.) and used at the third passage. The cells were seeded onto nitrous acid-precleaned, sterile glass microscope slides in 150-mm culture dishes, and cultured for 2 d before use in minimal essential medium-alpha medium without nucleosides (Flow Laboratories) supplemented with 50 U/ml penicillin, 50 μg/ml streptomycin, and 10% heat-inactivated fetal calf serum (Grand Island Biological Co., Grand Island, N. Y.).

For studies using intact cells, the slides were washed four times with Dulbecco's phosphate-buffered saline (13) containing 0.05% NaN_3, and drained. The area to be treated was marked by scoring a small circle on the underside of the slide with a diamond pencil. Then 50 μl of normal goat serum (NGS) diluted 1:20 was dropped onto the slide. 5 min later, 90 μl of either normal rabbit serum or antiserum, diluted 1:10, were added. After 30 min at 20°C, the slides were washed five times with buffer, drained, and 30 μl of 1:20 NGS was added. 5 min later, 90 μl of fluorescent antibody reagent (see above), diluted 1:8, was added. After 30 min at 20°C, the slides were washed five times, fixed with 2% paraformaldehyde in buffer at 20°C for 30 min, dehydrated in 90% ethanol for 15 min, and air-dried. Slides were viewed under 80% glycerine, 20% 0.2 M potassium borate, pH 8.5, in a Leitz Ortholux fluorescence microscope.

For studies using fixed cells, the slides were washed four times with buffer, fixed in 2% paraformaldehyde in buffer for 4 h, dehydrated with several changes of 90%

ethanol, and air-dried. The dried slides were then rewetted with 100 μl of 1:20 NGS, and then treated sequentially with serum and fluorescent antibody reagent, as described above.

Amino Acid Analyses

The amino acid composition was determined from duplicate protein samples hydrolyzed in 6 N HCl with 0.5% phenol added for tyrosine protection (2) and individually sealed under vacuum as described by Moore and Stein (27). Hydrolysis was carried out at 110°C for 20, 40, 72, and 120 h as described by Crestfield et al. (10) for extrapolating recoveries of individual amino acids to zero time for those labile amino acids, while those difficult to release were expressed as maximum values. Half/cystine content of these proteins was determined as cysteic acid according to the method of Moore (26). Amino acid analysis was carried out on the Durrum D-500 Amino Acid Analyzer (Durrum Instrument Corp., Sunnyvale, Calif.) modified for high sensitivity with the Mark II Data Analysis System.

RESULTS

Lectin Yield and Characterization

After homogenization of the various tissues, either all or substantially most of the lectin activity was recovered in the sedimentable fraction. The lectin could be released in soluble form by extraction with lactose, suggesting that the lectin was bound to the particulate material by its saccharide-binding sites.

The yield of lectin per kilogram wet weight of tissue varied considerably among the tissues sampled, as indicated in Table I; spleen was a particularly rich source of the lectin.

The isolated lectins were judged to be highly purified on the basis of Coomassie Blue-stained alkaline disc polyacrylamide gel electrophoresis (not shown) and SDS-polyacrylamide slab gel electrophoresis (Fig. 1), except calf thymus lectin,

TABLE I
*Yield of Galactose-Binding Lectins from Various Tissues**

Tissue	Particle-associated (lactose-extractable)	Soluble	Total
	mg	*mg*	*mg*
Calf spleen	43	5	48
Calf thymus	5.5	none detected	5.5
Calf liver	2.2	none detected	2.2
Calf heart	13	3	16
Chicken heart	4.2	none detected	4.2

* Yields are based on material specifically eluted from asialofetuin-Sepharose affinity columns, and are expressed as milligrams of lectin protein per kilogram wet weight of tissue.

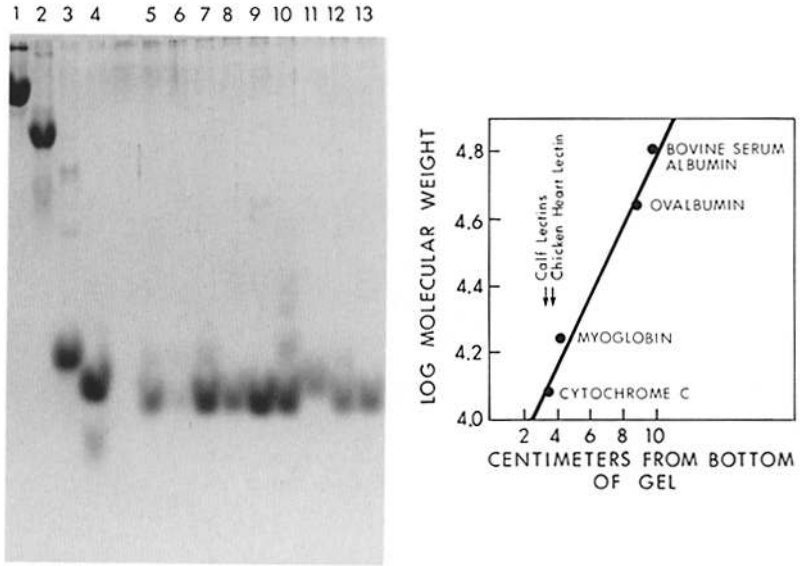

FIGURE 1 SDS slab gel electrophoresis. Electrophoresis was performed as described in Materials and Methods. Lane *1*, BSA; *2*, ovalbumin; *3*, myoglobin; *4*, cytochrome *c*. Lanes *5*, *6*, *12*, and *13*, calf spleen soluble lectin; *7*, calf spleen particle-associated lectin; *8*, calf heart soluble lectin; *9*, calf heart particle-associated lectin; *10*, calf thymus lectin; *11*, chicken heart lectin. *Inset:* Calibration curve used for estimating lectin molecular weights.

which gave several bands. The subunit molecular weights of the calf lectins were found to be virtually identical (~12,000 daltons), while that of the chicken heart lectin was clearly larger (~13,000 daltons) (Fig. 1). Gel filtration on Bio-Gel P60 gave a single peak of agglutinin activity, corresponding to apparent mol wt of 24,000 in the case of the calf lectins and 26,000 in the case of the chicken heart lectin, suggesting that in each case the agglutinin is a dimer. The major protein peak on Bio-Gel P60 corresponded with the agglutinin peak, while a small, variable amount of protein eluted at a position corresponding to 12,000 in the case of the calf lectins and 13,000 in the case of the chicken lectin; the minor peak persisted after affinity-chromatography repurification.

Analysis by isoelectric focusing revealed that both the soluble and lactose-extractable calf lectins from spleen and heart gave identical doublet (or, in other experiments, triplet) bands at pI ~4.8 (Fig. 2). The chicken heart lectin did not behave reproducibly on isoelectric focusing gels, but always focused in the acidic range (not shown).

The amino acid compositions of the purified lectins from chicken heart, calf heart, and calf spleen (lactose-extractable) are listed in Table II. The calf lectin compositions are remarkably similar to each other, while the chicken lectin is significantly different.

Antigenic Relationships among the Lectins

In a previous communication, de Waard et al. (12) demonstrated that soluble and particle-associated lectins from calf heart and lung all gave lines of identity on immunodiffusion plates. Similarly, calf spleen and thymus lectins gave lines of identity with the calf heart lectins on immunodiffusion plates (not shown), while the chicken heart lectin gave no band at all with anti-calf heart lectin serum. To further examine the antigenic relationships among these lectins, we performed radioimmunoassays. The results, illustrated in Fig. 3, demonstrate strong antigenic cross-reactivity among the calf lectins from heart, spleen, and liver (thymus was not tested). Since the anti-calf lectin serum used in these assays was directed against soluble heart lectin while the probes used were either spleen or heart particle-associated lectin, and since each antiserum was derived from a single rabbit, it is possible that some antigenic distinctions exist between the soluble and particle-associated forms of the lectin which our assays failed to pick up. Chicken heart lectin was distinctly different from the calf lectins. When chicken heart lectin was tested using anti-calf lectin serum and calf lectin probe, it was very weakly cross-reactive, while the reciprocal assay

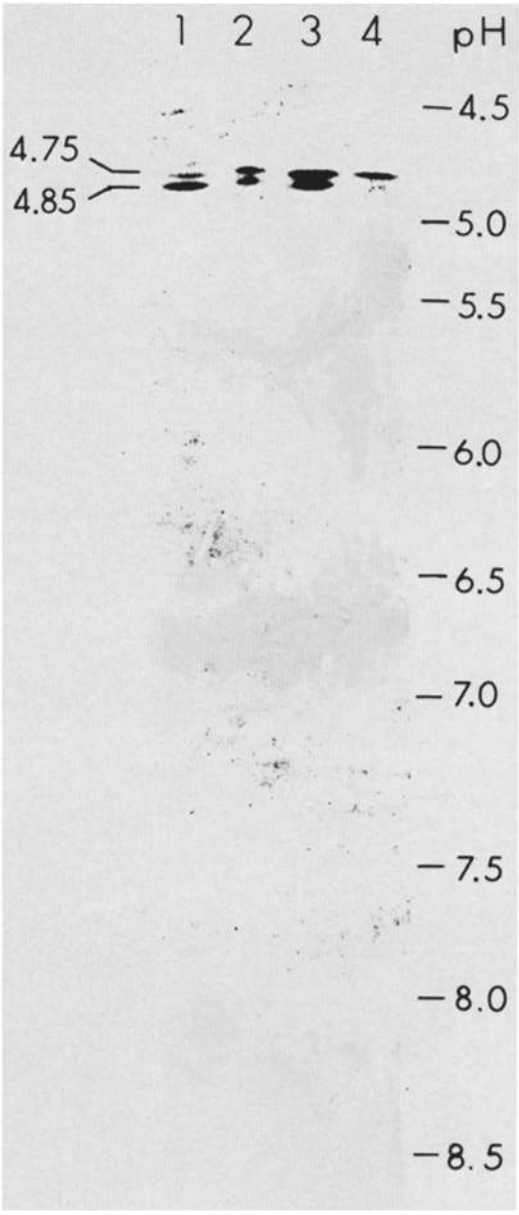

FIGURE 2 Isoelectric focusing patterns of calf lectins. Lane *1*, spleen particle-associated lectin; lane *2*, spleen soluble lectin; lane *3*, heart particle-associated lectin; lane *4*, heart soluble lectin.

TABLE II

*Amino Acid Compositions of Lactose-Extractable Lectins**

	Calf spleen	Calf heart	Chicken heart
Asp + $AspNH_2$	15.19	13.77	11.07
Thr	2.04	2.18	5.15
Ser	4.88	4.77	6.70
Glu + $GluNH_2$	8.74	8.61	10.77
Pro	4.39	6.03	5.63
Gly	8.15	8.95	9.10
Ala	9.43	9.54	3.93
Cys 1/2	4.52	3.89	2.29
Val	6.65	6.50	7.77
Met	0.00	0.15	1.45
Ile	3.39	3.51	3.80
Leu	10.16	9.62	7.68
Tyr	1.51	2.03	0.82
Phe	7.17	6.79	7.48
His	1.48	1.59	3.41
Lys	5.79	5.50	6.65
Arg	3.62	3.67	3.20
Try‡	2.89‡	2.90‡	3.08‡

* Expressed as No. of residues per 100 residues.
‡ Tryptophan values estimated.

using anti-chicken heart lectin serum and chicken heart lectin probe showed no cross-reactivity at all with calf lectins. Ricin, a plant lectin with similar saccharide-binding specificities (see below), showed no antigenic cross-reactivity with the vertebrate lectins in either assay system. Furthermore, neither lectin activity nor antigenically cross-reactive material could be found in fetal calf serum.

Lectin Binding to Rabbit Red Cells

Our binding studies demonstrate that the calf and chicken lectins have remarkably similar binding specificities. The apparent number of lectin-binding sites per rabbit erythrocyte for chicken heart lectin, calf spleen particle-associated lectin, calf heart particle-associated lectin, and calf heart soluble lectin is 2.5×10^6. As can be seen from Fig. 4, the various calf lectins and chicken heart lectin all compete with each other and with ricin for the same binding sites, but not with several other plant lectins tested. Furthermore, the order of efficacy of saccharide inhibitors is the same for both calf and chicken lectins (thiodigalactoside > lactose > α-methylgalactoside > β-methylgalactoside) as shown in Fig. 5. We have no explanation at present for the seemingly contradictory observation that α-methylgalactoside is a more potent inhibitor than β-methylgalactoside, while lactose and thiodigalactoside, both of which contain only β-linked galactosyl residues, are even more potent. Interestingly, however, the same

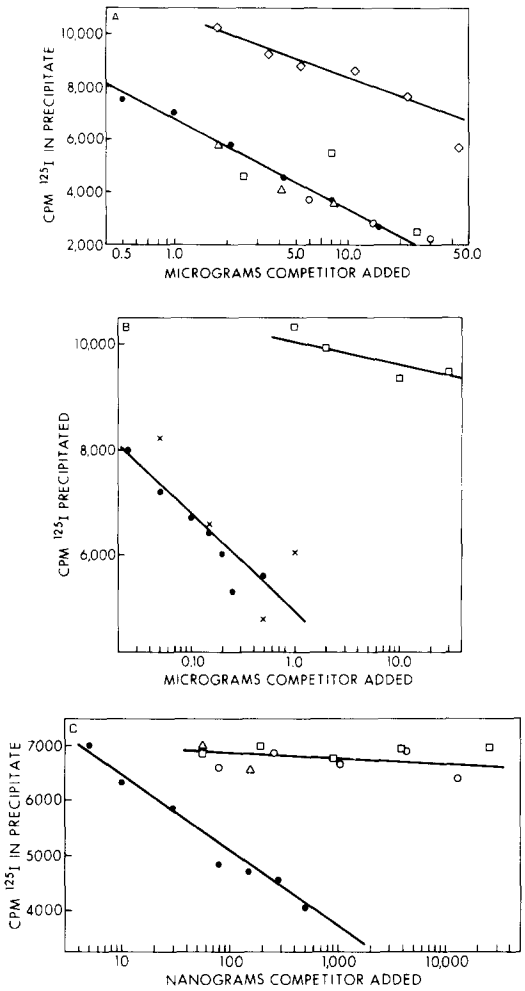

FIGURE 3 Radioimmunoassays. For procedural details, see Materials and Methods. (*A*) Rabbit anti-calf heart soluble lectin serum and ^{125}I-labeled calf heart particle-associated lectin probe. Competitors assayed: ●, calf spleen particle-associated lectin; △, □ two different calf heart particle-associated lectin preparations; ○, calf heart soluble lectin; ◇, chicken heart lectin. (*B*) Rabbit anti-calf heart soluble lectin serum and ^{125}I-labeled calf spleen particle-associated lectin probe. Competitors analyzed: ●, calf spleen particle-associated lectin; ×, calf liver lectin; □, ricin. (*C*) Rabbit anti-chicken heart lectin serum and ^{125}I-labeled chicken heart lectin. Competitors analyzed: ●, chicken heart lectin; ○, calf spleen particle-associated lectin; △, calf heart particle-associated lectin; □, ricin.

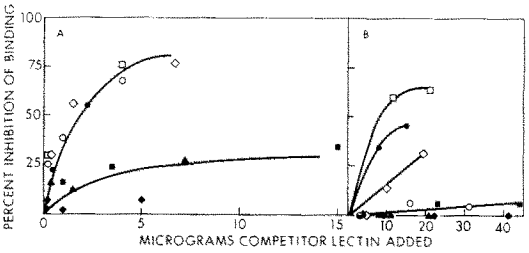

FIGURE 4 Inhibition of ^{125}I-lectin binding to rabbit cells by competing lectins. Inhibition assays were performed as described in Materials and Methods. Panel *A*: ^{125}I-labeled chicken heart lectin. Panel *B*: ^{125}I calf spleen particle-associated lectin. ◇, ricin; □, calf spleen particle-associated lectin; ●, chicken heart lectin; ▲, *Agaricus bisporus* agglutinin; ◆, *Phaseolus vulgaris* phytohemagglutinin E; ■, soybean agglutinin. Panel *A* only: ○, calf heart particle-associated lectin. Panel *B* only: ○, Concanavalin A.

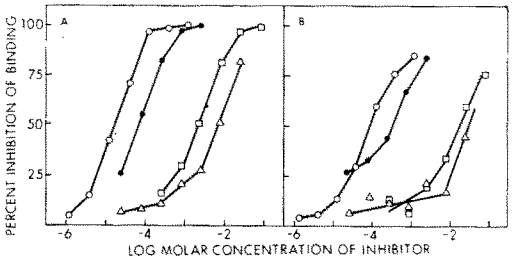

FIGURE 5 Inhibition of ^{125}I-lectin binding to rabbit red cells by saccharides. Inhibition assays were performed as described in Materials and Methods. Panel *A*: ^{125}I-labeled chicken heart lectin. Panel *B*: ^{125}I-labeled calf spleen particle-associated lectin. ○, thiodigalactoside; ●, lactose; □, α-methylgalactoside; △, β-methylgalactoside.

order of efficacy has been described by Nowak et al. (28) for a lactose-extractable lectin from embryonic chick muscle tissue, and may thus be idiosyncratic for vertebrate galactoside-binding lectins in general.

Despite their remarkable similarities with respect to binding specificity, the calf and chicken lectins differ considerably in their apparent binding avidities. The observed K_d of chicken heart lectin for rabbit erythrocytes was 4×10^{-8} M, while that of the calf lectins (heart soluble and particle-associated and spleen particle-associated) was 8×10^{-7} M. Neither trypsinization of the rabbit red cells nor varying the method of radioiodinating the lectins seemed to have any effect on the binding parameters; however, trypsinized rabbit red cells did agglutinate much more readily, and at lower lectin concentrations, than untreated cells.

Subcellular Localization of the Lectins

The tremendous antigenic cross-reactivity among the lectins from the calf tissues tested (see above) suggested that the lectin is ubiquitous, and that our anti-heart lectin antisera could therefore be used as reagents for the cellular localization of the lectin by immunofluorescence without necessarily using cells of heart origin.

Preliminary experiments using calf thymocytes teased from fresh thymus tissue demonstrated that most of the lectin was cytoplasmic but that some of it was on the extracellular face of the plasma membrane. However, many of the cells ruptured during the preparation of a single-cell suspension, and so it was possible that the cell surface lectin represented intracellular lectin from ruptured cells that had secondarily bound to surface receptor sites. We therefore performed immunofluorescence studies using cultured monolayers of calf kidney and chick embryo fibroblast cells, which could easily be manipulated without damaging the cells. Typical findings with the chick embryo fibroblasts are illustrated in Fig. 6. Fluorescence was uniformly distributed throughout the cytoplasm of fixed cells (but not in the nucleus), but no fluorescence was detected in unfixed cultures. These results indicate that the lectin is located primarily in the cytoplasm. Similarly, cellular fluorescence was observed in fixed bovine embryonic and postnatal kidney cells, but not in unfixed cultures, with one exception. Sometimes thin cytoplasmic strands were observed connecting two distal bovine cells, and these thin strands showed specific immunofluorescence in unfixed cultures (data not shown).

Some antigenic cross-reactivity was observed, in that chick embryo fibroblasts treated with anti-calf heart lectin serum fluoresced more brightly than those treated with control normal rabbit serum (Fig. 6*c*) but less brightly than those treated with anti-chicken heart lectin serum. This was consistent with our radioimmunoassay findings (see above).

DISCUSSION

Since the first report by Teichberg et al. (33), of the existence of β-galactoside-binding lectins in tissues of various vertebrate species, several laboratories have investigated such proteins. Lectins

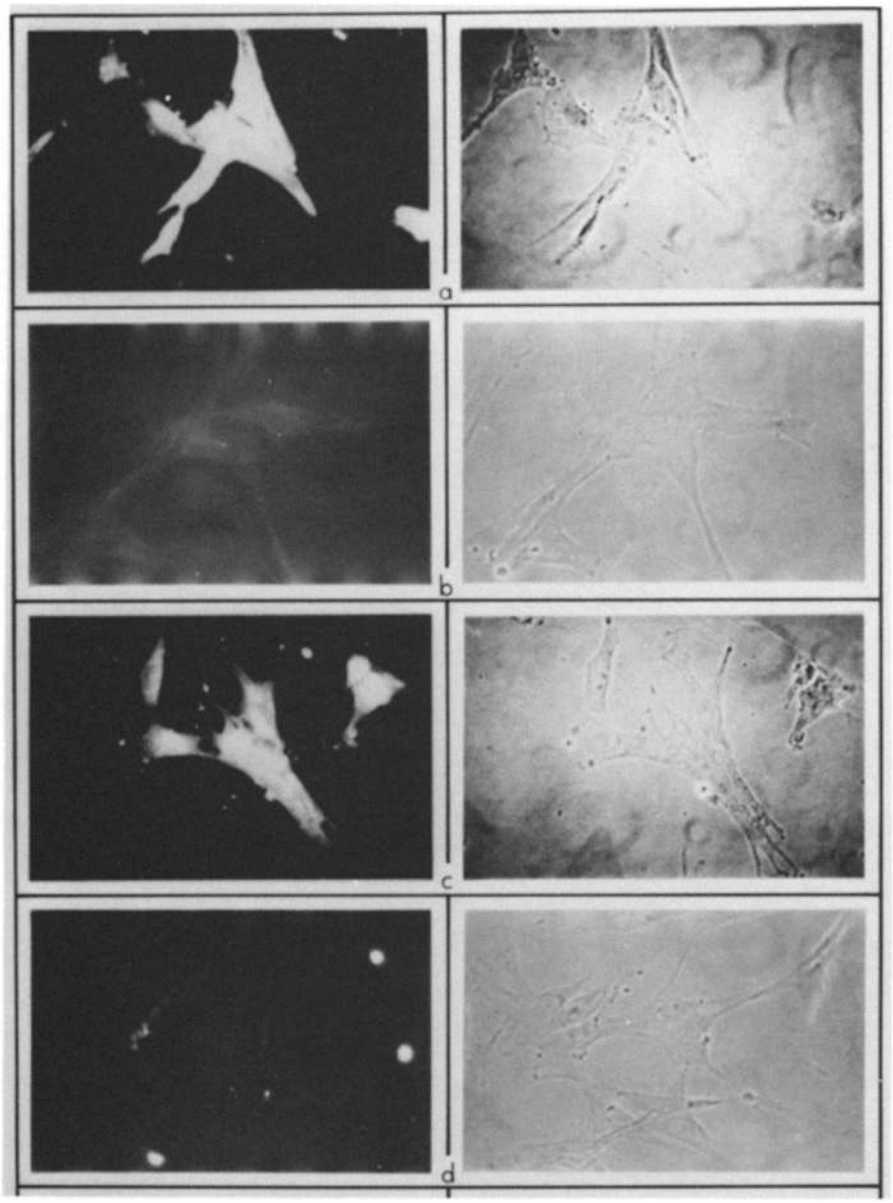

FIGURE 6 Cellular localization of lectin by immunofluorescence microscopy. These experiments were performed as described in Materials and Methods. Each field was photographed using both fluorescence optics (left) and phase contrast (right). The original magnification was 500. (*a*) Fixed, ethanol-treated chick embryo fibroblasts (CEFs) plus anti-chicken heart lectin serum. (*b*) Fixed CEFs plus normal rabbit serum (control). (*c*) Fixed CEFs plus anti-calf heart lectin serum. (*d*) Unfixed, intact CEFs plus anti-chicken heart lectin serum.

with properties remarkably similar to those of the "electrolectin" isolated from electric eel electric organs (33) have been reported from sources as diverse as embryonic chick muscle (11, 28) and calf hearts and lungs (12). The properties which these lectins all appear to share include: a stringent requirement for thiol-reducing groups; an apparent specificity for terminal β-galactose resi-

dues such as those of thiodigalactoside or lactose, yet a paradoxically marked preference for α-methylgalactoside over β-methylgalactoside; an acidic pI; a dimeric structure comprised of two polypeptide chains ranging in apparent molecular size from 12,000 to 17,000 daltons; and an intracellular, cytoplasmic localization.

From the the work of de Waard et al. (12) and the data presented in this report, it is clear that the lectins from various tissues of the calf (heart, lung, liver, spleen, and thymus) are very closely similar, and probably identical. They exhibit the same apparent size on gel permeation chromatography and SDS-PAGE; they all have the same binding specificity; they are antigenically cross-reactive, and give lines of identity on both immunodiffusion and radioimmunoassay; and they show identical isoelectric focusing patterns. In addition, the heart and spleen lectins have remarkably similar amino acid compositions. In contrast, the lectin from chicken hearts, although similar in general characteristics, is clearly different from the calf lectin(s). It exhibits a slightly larger molecular size, and it is antigenically distinct. These findings suggest that the vertebrate lectins are species-specific rather than tissue-specific. This is in agreement with the finding by Kobiler et al. (22) that lectins from a number of embryonic chick tissues appear identical.

Our immunofluorescence studies revealed that the lectin is present primarily in the cytoplasm of cells. The question of whether any lectin is present on cell surfaces is of interest with respect to possible functions of the lectin. We did not observe any detectable lectin at the cell surface, except under circumstances where there was a strong likelihood that lectin was released from damaged cells and subsequently bound to cell surface receptors; however, this does not exclude the possibility that some lectin is normally present on the cell surface but in amounts too low to be detected by our methods. We did, however, observe the lectin on thin cytoplasmic strands which connected distal bovine cells; the significance of this is not clear. In a similar investigation, Nowak et al. (28) demonstrated that most of the β-galactoside-specific lectin of chick embryo myoblasts undergoing fusion in vitro is located in the cytoplasm; however, they did observe some lectin at the cell surface. Furthermore, Gremo et al. (18) examined dissociated tectal cells for surface lectin and found that fewer than half of the cells in their preparations were positive; however, they could not exclude the possibility that some lectin had leaked out of damaged cells.

On the basis of our data, we conclude that, in the intact cell, the lectin is located predominantly intracellularly. In tissue homogenates, the bulk of the lectin is associated with particulate material (probably membranous), and can be recovered in soluble form by extraction with lactose, Triton X-100 (12), or high ionic strength (11). We believe that this is most likely an artifactual situation resulting from the release of intracellular lectin during homogenization and the subsequent binding of the lectin to receptors on the membrane fragments. We would like to note that the distinctions reported previously from this laboratory (12) between the particle-associated and soluble forms of the calf heart lectin have not been reproducible in the present study.

It has been suggested by other investigators that these lectins might play a specific role in myoblast adhesion and fusion during embryonic development (11, 15, 21, 28). However, our findings, plus the remarkable ubiquity of these lectins, lead us to believe that the lectins may be responsible for some (as yet unknown) cellular or physiological function which is not specific for any particular organ or tissue.

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