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Isolation of a Developmentally Regulated Lectin from Chick Embryo

Malcolm J. Pitts and David C.H. Yang

Department of Chemistry
Georgetown University
Washington, D.C. 20057

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SUMMARY

A lectin is isolated from the microsomal fraction of chick embryo kidney after initial extraction with 1 M urea and 0.3 M lactose. To exhibit hemagglutination activity, the lectin in the microsomal fraction requires prior activation by solublization with deoxycholate or by treatment with trypsin, chymotrypsin or phospholipase c. The lectin is partially purified by hydrophobic interaction chromatography about 200 fold from the microsomal fraction. The lectin binds strongly to de-sialated embryonic carbohydrates and shows low affinity toward glucosamine, galactosamine and mannosamine, as judged by the inhibition of hemagglutination. Comparison of the lectin activity from kidneys of embryos at different ages shows that the lectin is developmentally regulated.

Carbohydrate binding proteins known as lectins have been mainly isolated from plant seeds and have been extensively used as cytochemical probes to study the functional roles and dynamics of cell surface carbohydrates of vertebrates (see ref. 1 for a review). Lectins exhibit a number of activities, notably cell agglutination and lymphocyte transformation. In embryonic cells of vertebrates, cell surface carbohydrates have been thought to play a significant role in intercellular recognition, as suggested by inhibition studies (2) and chemical modification of cell surface carbohydrates (3, 4). The isolation of lectins from vertebrates (5, 6) opens the possibility that lectins may mediate cellular interactions by recognizing specific cell surface carbohydrates. This possibility is substantiated as an increasing number of vertebrate lectins have been isolated and found to be membrane-bound (7-10).

During our search for a better isolation procedure for the cell aggregating factor (11), the trypsin digest of several embryonic tissues was found to contain both hemagglutination and cell aggregation promoting activities (12). The two activities failed to be resolved by a number of protein purification procedures, probably due to the heterogeneity introduced by the initial trypsinization. To further characterize the embryonic lectin, we have isolated the lectin without prior trypsinization. In this communication, we report the preliminary results of the isolation and some unusual properties of the new embryonic lectin.

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MATERIALS AND METHODS

Materials: White Leghorn chick embryos were obtained from Truslow Farms (Chestertown, Md.). Simple saccharides were purchased from Sigma or Pfanstiel Laboratories. Crystalline trypsin, phospholipase c, pronase, Clostridium perfrigens neuraminidase (14 units/mg), and aprotinin were obtained from Sigma. Other chemicals were analytical grade or the purest form available from standard sources.

Analytical Methods: Protein concentration was determined according to Lowry et al (13) using bovine serum albumin as the standard or by the method of Warbury and Christian (14). Carbohydrate concentration was determined by the phenol-sulfuric acid method using lactose as the standard (15). The sedimentation coefficients were determined by sucrose gradient centrifugation (16) using bovine catalase (11.3 S), ovalbumin (3.55 S), soybean trypsin inhibitor (2.3 S) and cytochrome c (1.83 S) as standards. The Stokes radius of the lectin was determined by gel filtration according to Nozaki et al (17) using β-galactosidase (69 Å), β-lactoglobulin (27.4 Å), soybean trypsin inhibitor (22.5 Å) and cytochrome c (17.4 Å) as standards (18). The data was analyzed according to Ackers (19).

Hemagglutination Assay: Lectin mediated hemagglutination was used to assay the lectin activity (20). The trypsinized rabbit erythrocytes were prepared according to Lis and Sharon (21). The hemagglutination activity was determined using 2-fold serial dilutions of the lectin in microtiter U-plates. Each well contained 25 µl of a 4% erythrocyte suspension and 25 µl of the lectin diluted with phosphate buffered saline (PBS contained in 1 l, 8.0 g of NaCl, 0.2 g of KCl, 0.2 g of KH_2PO_4 and 0.15 g of Na_2HPO_4, pH 7.2). The titer is the highest dilution of the lectin that visably agglutinated erythrocytes under these conditions. The inhibitory effects of saccharides on hemagglutination were determined from the decrease of the agglutination titer, at a given concentration of the lectin, in the presence of varying concentrations of the saccharide.

Preparation of the Lectin: Kidneys were dissected from 120 15-d-old embryos and hand-homogenized using a Thomas tissue grinder in calcium-magnesium-free medium containing 100 units of aprotinin at 4°C. The homogenate was centrifuged at 2,000 rpm for 10 min in a Sorvall SS-34 rotor at 4°C. The pellet was resuspended and incubated in PBS containing 1 M urea, 0.3 M lactose and aprotinin (100 units/ml) at 37°C for 30 min. The incubation mixture was then sedimented at 10,000 g for 10 min at 4°C. The supernatant from 10,000 g centrifugation was resedimented at 40,000 rpm for 1 h at 4°C. The microsomal fraction was then gently resuspended in 0.5% deoxycholate in PBS at 25°C. The resulting suspension was filtered through glass fiber filter (Whatman GF/A). The filtrate was dialyzed extensively against 0.05% deoxycholate in PBS at 25°C. The dialyzed filtrate was then chromatographed on a column of diaminohexyl-Sepharose. A typical chromatogram is shown in Fig. 1. The active fractions were pooled and dialyzed extensively against 0.05% deoxycholate. After a second hydrophobic interaction chromatography using diaminooctyl-Sepharose, the lectin was purified about 200 fold from the microsomal fraction.

Preparation of Embryonic Carbohydrates: The embryonic carbohydrates were prepared from tissues of 15-d-old chick embryos. Tissues were dissected from embryos and subsequently dissociated by incubating with 0.1% trypsin in CMF at 37°C for 30 min. After removal of single cells and tissue fragments by centrifugation, the supernatant was passed through a column of soybean-trypsin-inhibitor-Sepharose. The trypsin free supernatant was then digested with pronase (1 mg/ml) at 37°C for 2 d as described (22). Pronase was inactivated at 85°C for 10 min. After filtering through glass fiber filter (Watman GF/A), the carbohydrates were assayed for inhibition of agglutination. Neuraminidase treatment was carried out as described by Paulson et al (23). Neuraminidase was inactivated

at 100°C for 10 min and filtered by a glass fiber filter. Heat inactivated pronase, bovine serum albumin or neuraminidase had no effect on agglutination activity.

RESULTS

Activation and Isolation of the Embryonic Lectin: The homogenate of embryonic kidneys was initially extracted with buffer containing 1 M urea and 0.3 M lactose. After differential centrifugation, the microsomal fraction contained the latent lectin, while the soluble fraction contained the β-galactoside binding lectin (6; Pitts & Yang, unpublished results). Hemagglutination activity in the microsomal fraction could be demonstrated after partial solublization with 0.5% deoxycholate followed by filtration. The lectin in the filtrate was then chromatographed on a column of diaminohexyl-Sepharose. A typical chromatogram is shown in Fig. 1. Subsequent chromatography on diaminooctyl-Sepharose yielded a partially purified lectin with a specific activity of hemagglutination of 4×10^5 $mg^{-1}ml^{-1}$, which is comparable to highly purified animal lectins (6, 24).

The embryonic lectin had a Stokes radius of 44 Å and an unusually low sedimentation constant of 3.7 S (see Materials and Methods for experimental details).

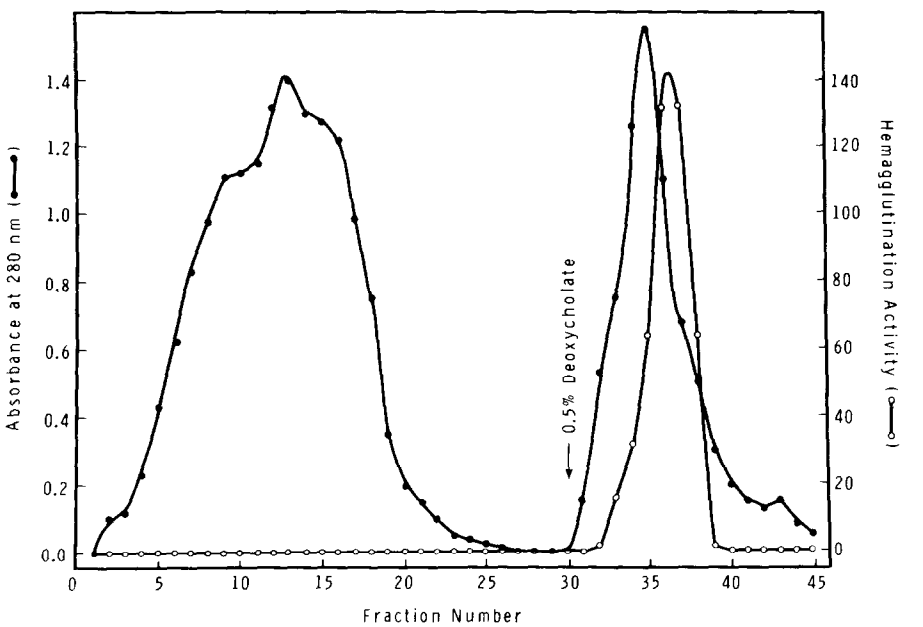

Figure 1: Hydrophobic interaction chromatography of the chick embryonic lectin. The dialyzed microsomal fraction (120 mg) from 120 15-1-old embryos was loaded to a column (1.8x5 cm) of diaminohexyl-Sepharose, pre-equilibrated with 0.05% deoxycholate in PBS. The column was eluted with the same buffer until the absorbance at 280 nm was below 0.05 and was then eluted with 0.5% deoxycholate in PBS. Fractions of 1 ml were collected and assayed for hemagglutination activity (—○—○—) and protein by absorbance at 280 nm (—●—●—). Recovery of hemagglutination activity was 600%.

Table I: Activation of the Embryonic Lectin in the Microsomal Fraction[a]

Treatment	Hemagglutination Titer	
	Kidney	Heart
PBS	0	0
0.5% Deoxycholate[b]	16	32
Trypsin[c]	16	32
Chymotrypsin[c]	16	32
Pronase[c]	32	32
Phospholipase[c]	32	32
Lipase[c]	0	0

a. For preparation of the microsomal fraction and assay of hemagglutination activity, see Materials and Methods

b. The microsomal fraction of embryonic kidneys or hearts was resuspended in PBS containing 0.5% deoxycholate followed by filtration.

c. The microsomal fraction, resuspended in PBS, was incubated separately with 1 mg/ml of each enzyme at 37°C for 30 min. At the end of incubation, proteases were inactivated by 1mM phenylmethylsulfonyl fluoride and phospholipase c and lipase were precipitated with 10% ethanol.

Since the low sedimentation constant could be resulted from a high axial ratio (25) or a high partial specific volume (26) due to the binding of deoxycholate or the presence of a carbohydrate moiety, the molecular weight of the lectin could not be reliably determined.

The following observations during the isolation of the embryonic lectin suggest that the lectin may be membrane- or receptor-bound in the microsomal fraction. The microsomal fraction, as suspended in PBS, did not exhibit any hemagglutination activity. The lectin was readily activated by dissolving the microsomal fraction in 0.5% deoxycholate. The lectin could also be activated by treatment with trypsin, chymotrypsin or phospholipase c (Table I). The enzymatically activated lectin was retained by a glass fiber filter and was resistant to further proteolytic treatment (e.g. 0.1 mg/ml of trypsin at 37°C for 30 min). Conversely, the deoxycholate solublized activity readily passed through a glass fiber filter and was completely inactivated by trypsin under the same conditions. Finally, the lectin bound strongly to the hydrophobic interaction gels and was effectively eluted off the column by deoxycholate with concomitant increase of hemagglutination activity.

Inhibition of Hemagglutination by Carbohydrates: To determine whether the hemagglutination was mediated through the binding of carbohydrates, inhibition of the lectin-mediated hemagglutination by simple saccharides was examined. As

Table II: Inhibition of Hemagglutination

Simple Saccharides	Concentration that inhibit agglutination by 50% (mM)	
Galactosamine	10	
Glucosamine	10	
Mannosamine	10	
Others[a]	>180	
Embryonic Carbohydrates[b]	Concentrations that inhibit agglutination by 50% (µg/ml)	
	-Neuraminidase	+Neuraminidase[b]
Kidney	>37	2.3
Heart	>70	0.65
Retina	>31	3.5
Brain	>47	2.2

a. Other saccharides which did not inhibit agglutination at concentrations up to 180 mM, include galactose, glucose, mannose, lactose, sucrose, maltose, thiodigalactoside, N-acetyl-galactosamine and N-acetyl-mannosamine.

b. See Materials and Methods for the preparation of embryonic carbohydrates and the treatment with neuraminidase.

shown in Table II, none of the simple saccharides except glucosamine, mannosamine or galactosamine at 10mM inhibited hemagglutination. The hemagglutination was not due to simple electrostatic interaction, since sodium chloride at up to 0.4 M did not inhibit hemagglutination. These properties are similar to fibronectin (8) and a platelet agglutinin (9) but different from other animal lectins (5, 6, 24, 27).

The high affinity of the lectin toward carbohydrates was demonstrated using embryonic carbohydrates. As shown in Table II, very low concentrations of carbohydrates from various embryonic tissues, after neuraminidase treatment, inhibited hemagglutination. Although the embryonic carbohydrates might contain non-carbohydrate materials, inhibition of hemagglutination only after the removal of sialic acid strongly suggests that carbohydrate moieties account for the observed inhibition. In this regard, the lectin is very similar to the hepatic lectin reported by Ashwell and coworkers (7).

Developmental Regulation of the Lectin: The regulation of the lectin during development was demonstrated by comparing the hemagglutination activity in kidneys of embryos at various ages. The lectin was purified from the microsomal fraction by hydrophobic interaction chromatography before comparison, in an attempt to remove bound receptors or endogenous inhibitors. To eliminate the possibility that the obser ved time course might be a function of differences in the extraction procedures or

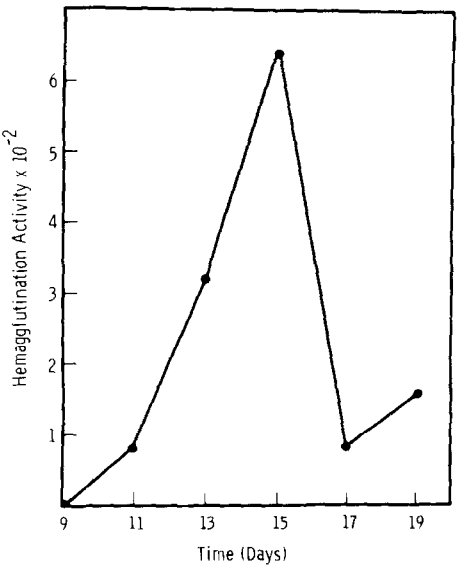

Figure 2: Developmental regulation of the embryonic lectin in kidneys. The embryonic lectin in the microsomal fraction of kidneys was purified from six embryos at each developmental age and compared for the total hemagglutination activity. See text for more details.

in the agglutinability of the erythrocyte preparation, we concurrently purified the lectin from embryos at various ages by the same procedure, used freshly prepared solutions for extraction and assayed them with the same erythrocyte preparation on the same day. As shown in Fig. 2, the lectin appeared in 9-d-old embryos, increased over 100 fold at 15 d and sharply declined at 17 d. The specific activity of the lectin also exhibited similar age dependency. Whether the second increase of the hemagglutination activity in 19-d-old embryos was contributed by a structurally different lectin from earlier ages is not known at present, although the sugar binding properties remained the same. It should be noted that the β-galactoside binding lectin also showed developmental regulation in chick embryos (28).

DISCUSSION

In this communication, we report the preliminary results of the isolation and properties of a new embryonic lectin. Many unusual characteristics of the lectin were observed. Prior activation of the lectin is required through solublization with deoxycholate or treatment with hydrolytic enzymes. The lectin binds tightly to hydrophobic interaction gels and is recovered by deoxycholate in a more than quantitative yield. The lectin binds strongly to de-sialated embryonic carbohydrates but shows little affinity toward simple saccharides. Finally, the lectin is apparently regulated during development.

The embryonic lectin was also isolated from hearts and brains of chick embryos by the same procedure, and showed similar sugar binding properties (data not shown). The activity in embryonic hearts and kidneys was four times higher than that in brain.

A lectin with similar sugar binding properties was also isolated from the trypsin digest of embryonic tissues together with cell aggregating activity, which promoted the sizes of 3-hr aggregates of homologous embryonic cells. Since the distribution of the lectin in different tissues and its age dependency during development (Pitts & Yang, unpublished results) did not correlate with those of cell aggregating activity (11), these two activities are likely due to different molecular entities. However, because of the strong binding of the lectin to embryonic carbohydrates, the involvement of the lectin in different aspects of cellular recognition remains an open possibility.

The lectin may be an integral membrane protein as suggested by the occurrence of the lectin and the microsomal fraction, the nature of the activation of the lectin and the strong binding to hydrophobic interaction gels. In this regard, the embryonic lectin is similar to the liver lectin (7). Similarly, both lectins bind to de-sialated carbohydrates. However, they differ in sugar binding properties and in that the embryonic lectin required prior activation and was solublized with deoxycholate but not with Triton X-100.

In view of the importance of carbohydrates in intercellular (2-4) and more recently intracellular recognition (29), the isolation of the embryonic lectin should provide an additional tool for further examination of the involvement of lectins in cellular recognition. Present isolation of the lectin without prior trypsinization provides the intact polypeptide chain of the lectin for structural studies. The embryonic lectin is being purified to homogeneity in our laboratory for structural and functional studies by direct chemical and immunochemical approaches.

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