76. Chick Embryonic Cartilage has a Developmentally Regulated Lectin Specific for Galactose-Containing Saccharides

By Tatsuya YAMAGATA and Tomoko NISHIWAKI

Mitsubishi-Kasei Institute of Life Sciences,
11 Minamiooya, Machida-shi, Tokyo 194

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Accumulating evidence has shown that proteins which bind specifically to carbohydrates and agglutinate erythrocytes are found not only in plants, but also in animal tissues. In the development of the chick embryo, the occurrence of carbohydrate binding proteins (lectins) in muscle,¹⁾⁻⁴⁾ brain, heart and liver,^{5),6)} retina and spinal cord⁷⁾ was reported, although the functions of these lectins have not yet been defined. During the study on a tissue antagonist of interferon, Chany-Fournier *et al.*⁸⁾ have shown that mouse costal cartilage, obtained from newborn or adult Swiss mice, contains a lectin-like substance, the activity of which is inhibited by some sugars. In this report we describe the occurrence of a lectin specific for galactose-containing saccharides in chick embryonic sterna, and we demonstrate that the activity of this lectin changes during development of the embryo.

Fertilized eggs of the White Leghorn chicken were obtained from a local hatchery and incubated at 38°C until use. At the time indicated, chick embryonic sterna were dissected and cleaned off the surrounding perichondrium in Ca^{++} , Mg^{++} free Tyrode's solution. The sterna were immediately placed in 20-fold volume of 0.3 M lactose/0.15 M NaCl/20 mM Na-phosphate buffer at pH 7.2/4 mM 2-mercaptoethanol at 0°C and treated in a Polytron (made by Kinematica GmbH, Switzerland) for 60 sec at top speed. The homogenates were centrifuged at 100,000 g for 30 min at 4°C. The supernatant solution was dialyzed against several changes of phosphate buffered saline containing 4 mM 2-mercaptoethanol (MEPBS). The dialyzate was concentrated by the use of PM-10 membrane (Amicon Co.) and assayed for lectin activity by hemagglutination, using trypsinized rabbit erythrocytes.⁶⁾

The specific activity of the erythrocyte agglutinating factor was found to be highest in extracts prepared from the 11 and 12 day old chick embryo sterna (Fig. 1). Thereafter the specific activity decreased with time and at the time of hatching reached one tenth the

Fig. 1. Specific activity of the factor in chick embryo sternal extracts which causes hemagglutination as a function of developmental age (●). Activity is also expressed per sternum (○). The results obtained from 12 to 14 day chick embryo sterna are the average of studies with two to three separate extracts. Others are based on the average of several determinations of one extract.

Agglutination activity was determined in microtiter U type plates (Linbro Co.), using serial twofold dilutions of the sample in MEPBS. The reaction mixtures were composed of 25 μ l of the diluted sample, 25 μ l of PBS or various concentrations of sugars in PBS and 25 μ l of 2% suspension of trypsinized rabbit red blood cells in PBS. A unit of lectin activity is defined as the reciprocal of the highest dilution of the sample which gives detectable erythrocyte agglutination. Results of the agglutination assay are expressed in units per mg of total protein in the sample added to the reaction mixture. Protein was determined by a modified micromethod of Lowry,⁹ using bovine serum albumin (Sigma, fraction V powder) as a standard.

level of the 11 day sternal extract, which was the earliest tested. When erythrocyte agglutinating activity is expressed per sternum, the activity showed a peak in the 14 day chick sternal extract (Fig. 1). Decrease of the specific activity at 14 day chick sternal extract could be elucidated as the overwhelming increase of the extractable protein which has nothing to do with the lectin activity. The maximal increase of body weight of chick embryos is reported to occur at day 14.¹¹ Kobilier and Barondes,⁵ and Eisenbarth *et al.*⁷ expressed the hemagglutinating activities obtained from chick embryonic heart, brain, liver, muscle, spinal cord and retina in a specific activity. If

they express the activities in a total activity, the day which gives the maximal total activity could be different from those assigned for the maximal specific activity.

To evaluate the erythrocyte agglutinating factor, we studied the effect of a number of mono- and disaccharides as inhibitors of hemagglutination. Table I summarizes the concentration of various sac-

Table I. Inhibition by saccharides of hemagglutinating activity

Saccharides	Concentration for 50% inhibition (mM)	Saccharides	Concentration for 50% inhibition (mM)
Lactose	0.16	Fucose	100
Melibiose	2	Rhamnose	>100
Galactose	10	Maltose	>100
Galactosamine	33	Gentiobiose	100
Glucosamine	100	Cellobiose	>100
N-Acetylglucosamine	100	Methyl- α -D-Glucoside	100
N-Acetylgalactosamine	100	Methyl- β -D-Glucoside	100
Glucose	100	Methyl- α -D-Mannoside	>100
Mannose	100	EDTA	>5
Arabinose	33	Glucuronic acid	10
Xylose	>100	Galacturonic acid	7
Ribose	>100	Na ₂ SO ₄	100

Inhibition studies by saccharides of the hemagglutinating activity were carried out by using the sternal extract obtained from 14 day old chick embryos. During the course of these studies, we noticed that melibiose purchased from Fluka AG was contaminated by a lectin activity, and it was necessary to purify the sample on a column of Sephadex G-100 to obtain melibiose free from the lectin activity. This contaminating activity was slightly inhibited by fucose, mannose, glucosamine and galactosamine but not by glucose, galactose, xylose and N-acetylgalactosamine even at 180 mM. Since raffinose obtained from Fluka AG was not contaminated by a lectin activity, it is likely that the contaminating lectin activity has its origin in yeast which has been used to prepare melibiose from raffinose.

charides which caused half maximal inhibition of erythrocyte agglutination by the sternal extract.

Among various saccharides tested, those containing galactose inhibited agglutination. Lactose (D-galactosyl 1 $\xrightarrow{\beta}$ 4 glucose) was found to be the most potent inhibitor, while melibiose (D-galactosyl 1 $\xrightarrow{\alpha}$ 6 glucose) inhibited 10 times less effectively. Glucuronic acid and galacturonic acid were also found to be inhibitors of the hemagglutinating factor. Galactosamine and arabinose were weak inhibitors. Glucosamine, N-acetylglucosamine, N-acetylgalactosamine, glucose, mannose, fucose, gentiobiose, methyl- α -D-glucoside, methyl- β -D-glucoside,

side, Na_2SO_4 showed half maximal inhibition at 100 mM. Xylose, ribose, rhamnose, maltose, cellobiose, methyl- α -D-mannoside did not inhibit even at 100 mM. EDTA was shown to inhibit the hemagglutination of the cell surface protein fibronectin at 0.01 mM,¹⁰⁾ while EDTA did not inhibit hemagglutination by the sternal extract even at 5 mM.

The sternal lectin was found to be very sensitive to lactose (half maximal inhibition at 0.16 mM) and ten times less sensitive to melibiose (half maximal inhibition at 2 mM). The saccharides which inhibit hemagglutination by the sternal extract are similar to those reported for muscle lectin,⁴⁾ and for retina and spinal cord lectins.⁷⁾ However, the muscle lectin is not inhibited by melibiose, and the concentration of melibiose required to inhibit retina and spinal cord lectins is roughly ten times that required for the sternal lectin. Based on the inhibition studies of the lectin by saccharides, it is likely that the lectin activity of chick embryonic sterna is expressed by a lectin different from those described for muscle,⁴⁾ retina and spinal cord.⁷⁾

Though nothing is known about the role of chick embryonic lectins at present, we believe that they are involved in cell differentiation. It may be noteworthy that carbohydrate binding proteins have been shown to participate in recognition and uptake of specific glycoproteins by animal cells.¹²⁾⁻¹⁴⁾

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