Identification of the major mannose-binding proteins from chicken egg yolk and chicken serum as immunoglobulins

(affinity purification/binding specificity)

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ABSTRACT Chicken egg yolk contains a mannose-binding protein that could be purified with a modification of the procedure of affinity chromatography and gel filtration used for chicken serum mannose-binding protein. The yolk protein was indistinguishable from the serum protein with respect to apparent molecular masses (169 ± 7 kDa), subunits (74 kDa and 27 kDa, in approximately 1:1 ratio) produced after denaturation in the presence of mercaptoethanol, immunoreactivity with antibody against the chicken serum mannose-binding protein, amino acid composition, pH optimum for binding, calcium independence of binding, sugar-binding specificity, and specific-binding activity. Moreover, the chicken mannose-binding proteins cross-reacted with γ-chain-specific antibody against chicken IgG. The binding proteins were identified as IgGs by several other criteria: (i) identical electrophoresis pattern when subjected to reducing and non-reducing NaDodSO4/polyacrylamide gel electrophoresis, (ii) cochromatography on a Fractogel TSK HW-55(F) column, (iii) similar amino acid composition, and (iv) isolation of mannose-binding protein from purified serum IgG at a yield comparable to whole serum. These results support the notion that the mannose-binding proteins from the chicken serum and egg yolk are similar, if not identical, and are a subset of chicken IgG.

Recently, we have reported the presence of mannose-binding activity in chicken serum and the isolation of its major component (1). This protein is quite different from chicken liver N-acetyl-d-glucosamine (GlcNAc)-binding protein (2–4), the chicken liver mannan-binding proteins (5), and the known mammalian serum mannose-binding proteins (6, 7). Since mannose-binding activity was present in the sera of adult and juvenile chickens, it was of interest to search for mannose-binding activity in chicken eggs. Chicken eggs contain high concentrations of proteins, many of which are also known to exist in serum (e.g., refs. 8 and 9). Indeed, mannose-binding activity was detected in the white and yolk of the egg and was isolated by a procedure similar to that applied to the isolation of the major mannose-binding protein from chicken serum (1).

Comparison of the structural and immunochemical properties of the mannose-binding proteins from chicken egg with that from chicken serum revealed that they are indistinguishable. Moreover, the presence of heavy and light subunits cross-linked by disulfide bridges led us to probe possible similarities between these mannose-binding proteins and IgG. The evidence we have accumulated indicates that the mannose-binding proteins from chicken serum and egg are really a subset of IgG.

The materials and methods are described in this section.

MATERIALS AND METHODS

Materials. The materials and methods were obtained from the indicated sources: frozen chicken serum (nonsterile, thrombolyzed), chicken serum IgG, and goat antiserum against chicken IgG (Fc specific) (Pel-Freez); protein standards for NaDodSO4/polyacrylamide gel electrophoresis (NaDodSO4/PAGE) and porcine IgG (Sigma); agarose LE, Gel Bond films (Marine Colloids, FMC, Rockland, ME); Fractogel TSK HW-55(F) (Pierce); DEAE Affi-Gel blue, NaDodSO4, and acrylamide (Bio-Rad); and sodium p-nitrophenolate (Eastman Organic Chemicals, Rochester, NY). Yolk IgG (10) was a generous gift from T. Roth (University of Maryland Baltimore County). White Leghorn hen eggs were locally procured at a grocery store. Man4-AI-lysozyme, Man43-Al-BSA (where BSA = bovine serum albumin), Man33-Al-BSA, L-Fuc28-Al-BSA, and GlcNAc43-Al-BSA were prepared as reported (11). Man-BSA-Sepharose CL-6B was prepared by first coupling BSA to Sepharose CL-6B (12) at 3 mg of BSA per ml of settled gel and then attaching ~20 mol of mannose per mol of BSA by amidination (11). For the coupling of BSA to Sepharose CL-6B, pyridine borane at 30 mM was used instead of NaCNBH3 (13). Rabbit antiserum against CS-II (where CS = prefix used to indicate chicken serum as the source of binding protein) was prepared as described (1).

Analytical Methods. The standard binding assay was based on the modified version (3) of the ammonium sulfate precipitation method (14) in which 125I-labeled Man33-Al-BSA and 125I-labeled Man43-Al-lysozyme were used as ligands. 125I-labeled Man42-Al-lysozyme was used as ligand in addition to the 125I-labeled Man33-Al-BSA because its assay background was typically lower by a factor of 2–5 than the BSA neoglycoprotein at any given dose. The labeled ligand (100 nM) was shaken with samples containing binding protein for 15 min at 25°C. The binding protein and bound labeled ligand were precipitated using 45% saturated ammonium sulfate and the precipitate was collected on Whatman 934-AH filter discs. The radioactivity in the collected precipitate was measured to quantitatively bound ligand. One unit of activity was defined as the quantity of binding protein that binds 1 ng of the labeled ligand, and the specific binding activity was defined as binding units/µg of protein. The pH dependency of yolk mannose-binding protein (CY-II, where CY = prefix used to indicate chicken egg yolk as the source of binding protein) binding to 125I-labeled Man33-Al-BSA was determined by a previously described method (1).

Abbreviations: BSA, bovine serum albumin; GlcNAc-Al-protein, neoglycoproteins to which n mol of thioglycosides have been attached by amidination using 2-imino-2-methoxyethyl 1-thioglyco- sides; CS, prefix used to indicate chicken serum as the source of binding protein; CY, prefix used to indicate chicken egg yolk as the source of binding protein.

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NaDodSO₄/PAGE was carried out as described by Laemmli (15). For reducing gels, protein samples were boiled for 3 min in 63 mM Tris-HCl (pH 6.8) containing 3% (wt/vol) NaDodSO₄, 10% (vol/vol) glycerol, 5% (vol/vol) 2-mercaptoethanol, and 0.01% (wt/vol) bromophenol blue. Mercaptoethanol was omitted when preparing samples for nonreducing gels. The reduced protein samples were electrophoresed in a 12.5% (wt/vol) acrylamide gel and the nonreduced samples were electrophoresed in a 6% (wt/vol) acrylamide gel. The gels were stained with Coomassie blue G-250 for visualization of protein bands (16).

Agarose gels for double immunodiffusion (17) were prepared by pouring 9 ml of hot 15% (wt/vol) agarose solution in 20 mM Tris-HCl, pH 7.4/0.15 M NaCl on a 5 × 7.5 cm glass microscope slide.

Protein concentration was determined by fluorescein assay after base hydrolysis of samples (18) using porcine IgG as standard. Iodination of ligands was by a chloramine-T method (19) as modified by Krantz et al. (20). Amino acid analyses were done as described (21).

RESULTS

Purification of Mannose-Binding Protein from Yolk. Egg yolks (270 ml from one dozen hen eggs) were gently blended and poured into 1200 ml of acetone at −20°C, and the mixture was stirred for 30 min in an ice bath. The precipitate was collected by suction, washed four times with 500 ml of acetone at −20°C, and dried under aspirator vacuum overnight at 4°C.

The remainder of the purification except for the TSK Fractogel column step was done at 4°C. Yolk acetone powder (114 g) was extracted with 1 liter of buffer I (10 mM Hepes, pH 7.8/1 M NaCl/20 mM CaCl₂/2H₂O) on a New Brunswick gyration shaker set at 150 rpm for 14 hr. The suspension was centrifuged at 27,300 × g for 60 min using a Sorvall RC-5B centrifuge. The supernatant was collected by passing through three layers of gauze and centrifuged as before. The supernatant from the second centrifugation was mixed with Man-AI-BSA-Sepharose CL-6B gel (108 ml) and gently agitated with a gyration shaker at 150 rpm for 1 hr. The gel suspension was then poured into a 2-liter graduated cylinder and the gel was allowed to settle for 5 hr. After the supernatant was siphoned off, the gel was packed into a 2.5 × 22 cm column and washed with 1.5 liters of buffer I until the absorbance at 280 nm was <0.005. The column was then eluted with 200 ml of 0.5 M GlcNAc in buffer I followed by 200 ml of 0.5 M mannose in buffer I. When absorbance of the eluted fractions at 280 nm was determined, a major peak was eluted with GlcNAc and a peak ~20% the size of the major peak was obtained with mannose elution. Mannose-binding assays indicated one major activity peak that was coincident with the 280 nm absorbing peak obtained with mannose elution. The remaining activity (~10%) was eluted with GlcNAc in a broad peak. The major mannose-binding activity peak (CY-M) was pooled and concentrated by ultrafiltration using an Amicon YM-10 membrane.

The concentrated CY-M fraction was subjected to gel filtration at room temperature (~21°C) on a column of Fractogel TSK HW-55(F) (2.5 × 93 cm) equilibrated in 20 mM Tris-HCl, pH 7.1/0.1 M NaCl (data not shown). One major protein peak was obtained and pooled (CY-II). Table 1 summarizes the steps of purification, giving the yield after each step and the specific activities of CY-M and CY-II. The specific activities of CY-M and CY-II (Table 1) and the Fractogel TSK HW-55(F) profile (one peak) indicated that CY-M and CY-II were of comparable purity.

Mannose-Binding Activity in Egg White. Egg whites were gently blended and then fractionated by pH adjustment and ammonium sulfate precipitation: fraction I was precipitated at pH 5.8, and fractions II, III, IV, and V were precipitated with 0–40%, 40–50%, 50–63%, and 63–100% saturated ammonium sulfate at pH 4.6, respectively. Only fraction II reacted with antiserum against CS-II. Affinity chromatography of fraction II using the Man-AI-BSA-Sepharose produced an active peak with 0.5 M mannose elution. However, the binding protein yield was ~0.1 that obtained from the egg yolk.

Characterization of CY-II and Comparison with CS-II. Molecular mass and structural properties. NaDodSO₄/PAGE under nonreducing conditions gave an estimate of 169 ± 7 kDa for the molecular mass of CY-II and CS-II and showed coelecrophoresis of the yolk- and serum-binding proteins with IgGs from chicken serum and yolk (Fig. 1A). Reducing NaDodSO₄/PAGE revealed that CY-II and CS-II, like yolk and serum IgG, contained subunits with molecular masses of 27 kDa and 74 kDa (Fig. 1B). Furthermore, CY-II and CS-II coeluted with serum and yolk IgG standards from Fractogel TSK HW-55(F) (2.5 × 93 cm) and Bio-Gel A-1.5m (1.5 × 130 cm) columns. Analogous to the work of Leslie and Clem (22) with chicken 7.1S immunoglobulins and Wang et al. (1) with CS-II, CY-II probably consists of two heavy (74 kDa) and two light (27 kDa) chains.

Immunological properties. The immunological properties of CY-II were compared with CS-II, serum IgG, and yolk IgG by double immunodiffusion (Fig. 2). Antiserum against CS-II and chicken IgG (F₄ specific) strongly reacted with serum- and yolk-binding proteins and the IgGs from both sources. The precipitin lines for each of these proteins completely fused, indicating the formation of identical antibody–antigen complexes. The immunoprecipitation of the serum and yolk standard IgGs by the polyclonal anti-CS-II serum was expected since many epitopes would be shared in the constant regions of different IgG idiotypes.

Conditions for ligand binding. The optimal pH range (pH 5–9) for binding of CY-II to 125I-labeled Man₄-Al-lysozyme was very wide, like that found for CS-II. Binding activity of CY-II, like CS-II, was independent of calcium. When CY-II was dialyzed against EDTA-containing buffer to deplete calcium, its binding activity was neither impaired nor enhanced by the addition of either CaCl₂ or EDTA up to 20 mM.

Sugar-binding specificity. The sugar-binding specificity of CY-II was studied with neoglycoproteins and simple sugars using the inhibition assay technique as described (1). Various concentrations of the compound to be tested were added to the standard incubation mixture (500 μl) containing 125I-labeled Man₄-Al-BSA (90 nM) and bound ligand was determined by ammonium sulfate precipitation (see Materials and Methods). The concentrations required for 50% inhibition of binding were 1.6 nM (Man₄-Al-BSA), 14 nM (L-Fuc₂₃-Al-BSA), and 11.2 μM (GlcNAc₄-Al-BSA). The corresponding values for CS-II are 2.6 nM, 6.3 nM, and 6.5 μM, respectively, under similar conditions (1). The results indicated that CY-II, like CS-II, was specific for Man-Al-BSA and L-Fuc-

<table>
<thead>
<tr>
<th>Purification stage</th>
<th>Designation</th>
<th>Protein, mg</th>
<th>Specific activity, units/μg</th>
<th>Yield, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone powder extract</td>
<td>—</td>
<td>13,300</td>
<td>0.02</td>
<td>100</td>
</tr>
<tr>
<td>Affinity chromatography</td>
<td>CY-M</td>
<td>2.1</td>
<td>25.9</td>
<td>20</td>
</tr>
<tr>
<td>Gel filtration</td>
<td>CY-II</td>
<td>2.1</td>
<td>26.4</td>
<td>21</td>
</tr>
</tbody>
</table>

*Specific activity using 125I-labeled Man₄-Al-lysozyme as ligand.

Table 1. Purification of mannose-binding protein from chicken egg yolk
AI-BSA, although it showed some activity toward GlcNAc-AI-BSA.

Simple sugars, as expected, were much weaker inhibitors than the neoglycoproteins. D-arabinose and D-lyxose, two pentoses homomorphous with L-fucose and D-mannose, respectively, possessed inhibitory activity similar to D-mannose and L-fucose, which, when tested at 20 mM, inhibited 125I-labeled Manα4-linked AI-BSA binding by 46% and 32%, respectively. At comparable concentrations, glycodies showed stronger inhibition than the parent sugars. For example, 20 mM of D-galactose did not show any inhibitory activity, but 20 mM methyl α-D-galactopyranoside inhibited 125I-labeled Manα4-linked AI-BSA binding by 13%. It is noteworthy that an aromatic aglycon such as a p-nitrophenol group enhanced the inhibitory power of simple sugars. Indeed, sodium p-nitrophenolate alone was a good inhibitor, showing 28% inhibition at a concentration of 2 mM.

Amino acid composition. Table 2 indicates that the amino acid compositions (expressed in residues per 100 residues) of CY-II, CS-II, yolk IgG, and serum IgG were quite similar.

Isolation of Mannose-Binding Protein from Pure Chicken

Serum IgG. Chicken serum IgG, prepared from 100 ml of chicken serum (10), was applied to the Man-AI-BSA-Sepharose CL-6B column (2.5 × 22 cm) and eluted with 0.5 M mannose in buffer I. The yield and specific activity of CS-II obtained were comparable to those obtained when CS-II was isolated directly from serum (1).

Determination of IgG Concentration in Egg Yolk and White. A semiquantitative double immunodiffusion method was used to determine the IgG distribution in the egg (23). IgG was present in yolk at a minimum of 9 mg of IgG per ml and in white at <70 μg/ml.

DISCUSSION

A most interesting finding of this report was the identification of the chicken mannose-binding proteins as a subset of IgG. This is based on the following lines of evidence: (i) The mannose-binding proteins from chicken serum and egg yolk contain 74- and 27-kDa polypeptide chains, identical to the...
subunits of chicken serum and egg yolk IgGs (Fig. 1B). (ii) The mannose-binding proteins and IgGs showed identical migration when electrophoresed in nonreducing NaDodSO₄/PAGE (Fig. 1A) and in chromatography on columns of Fractogel TSK HW-55(F) (2.5 × 93 cm) and Bio-Gel A-1.5m (1.5 × 130 cm). (iii) Double immunodiffusion indicated that mannose-binding proteins from chicken serum and egg yolk and IgG from chicken serum and egg yolk were immunochemically indistinguishable (Fig. 2). Results from rocket immunoelectrophoresis also led to the same conclusion (data not shown). (iv) The mannose-binding protein could be isolated from a purified preparation of chicken serum IgG. The yield of mannose-binding protein was comparable to that from the whole serum when no intermediate isolation of IgG was involved. (v) The amino acid compositions of the binding proteins and IgGs were quite similar (Table 2).

The general occurrence of mannose-binding IgG in chicken serum was demonstrated by its presence in the plasma of nine out of nine individual chickens examined and in animals from three widely separated locales: Maryland (Dover Poultry, Baltimore), Arkansas (Pel-Freez, Rogers), and Illinois (kindly provided by Mark Kuhlenschmidt, College of Veterinary Medicine, University of Illinois at Urbana–Champaign).

Evaluation of the following criteria showed that mannose-binding protein from egg yolk (CY-II) was indistinguishable from that from serum (CS-II): molecular masses of the native proteins and subunits, immunochromatographical properties, amino acid compositions, optimal pH for ligand binding, calcium independence of binding, calcium-binding specificity, and specific binding activity (see Table 3). However, it is possible that subtle differences may exist between CS-II and CY-II that we have not as yet probed. Indeed, the carbohydrate compositions of hen serum and yolk riboflavin-binding protein were found to be different (9). Table 3 also illustrates the striking differences between the mannose-binding proteins and the liver GlcNAc-binding protein.

In our previous report on CS-II, it was shown that glycosides of (6-aminohexanamido)tris(hydroxymethyl)methane were more inhibitory than mannose itself and it was speculated that hydrophobic binding of aglycon in these glycosides caused a tighter binding than the parent monosaccharides (1). Similarly, p-nitrophenoxy glycosides and p-nitrophenoxyacetamide itself possessed significant inhibitory power for ligand binding to the yolk mannose-binding protein. In this regard, the mannose-binding protein resembles many lectins that have been found to contain hydrophobic binding sites, such as lima bean lectin (25, 26). However, they differ in that the carbohydrate and hydrophobic binding sites of the mannose-binding protein overlap to an unknown extent to allow hydrophobic compounds to inhibit carbohydrate binding, whereas the two sites in lima bean lectin are independent and noninteracting (25).

Since serum and yolk IgG concentrations are similar (27) and IgGs appear to be transported equally well from serum to yolk (10), the yield of mannose-binding IgG from each source may be similar. Indeed, mannose-binding protein was present in egg yolk at about 40% of the concentration (wt/vol) found in serum. From one dozen eggs (270 ml of yolk), containing 13.3 g of protein, 2.1 mg of CY-II was obtained, whereas 19.6 mg of CS-II was isolated from 1 liter of chicken serum that contained 40.6 g protein (1).

The egg white yielded ~0.1 the binding-protein compared to egg yolk when the same isolation procedure was used. The relatively low yield from egg white could be explained by the known presence of a large amount of mannose- and GlcNAc-terminated glycoproteins in egg white, such as ovalbumin (28, 29) and ovomucoid (30, 31). These glycoproteins might tightly associate with the egg white mannose-binding protein to prevent its binding to the affinity column. Alternatively, the yolk may be the major storage locus in the egg for the mannose-binding protein since egg IgG is segregated in the yolk (27) and the binding protein is most likely a subset of IgG. Low amounts of binding protein may be present in egg white due to the small amounts of IgG in the ovuduct secretions that become the egg white. Indeed, the concentration of IgG in egg white is <70 µg/ml compared to a minimum of 9 mg/ml in yolk. Since white is present at approximately twice the volume as yolk in the egg, ~50 times more IgG is present in yolk. Thus, one would expect ~0.02 the mannose-binding IgG in white compared to that found in egg yolk. However, 5 times this mass of binding protein was isolated from egg white. This may indicate that the distribution of the mannose-binding protein between white and yolk may be deviating from the general distribution pattern of IgG in the egg. Alternatively, a major portion of the mannose-binding protein in egg white may be IgA and IgM, two classes of antibodies known to be present in significant amounts in the egg white (27).

In a recent report on chicken liver mannose-binding proteins by Oka et al. (5), various fractions have been shown to contain subunits of 70, 41, 32, and 28 kDa in different ratios. One of their fractions (F-13-2) appears to contain a component resembling CS-II, perhaps as a result of trapped blood within their starting livers. However, the major components are distinct from CS-II and CY-II by several criteria, including subunit properties on NaDodSO₄/PAGE, calcium dependence of binding, and sugar specificity (ManNAc > mannose = L-fucose = GlcNAc, where ManNAc = N-acetyl-D-mannosamine). Interestingly, in another study a fraction of mannose-binding protein (A-I) from human serum could be adsorbed onto protein A-Sepharose CL-6B and immobilized IgG against human IgG, moved together with IgG throughout purification steps, and its binding activity was calcium independent (7).

### Table 3. Comparison of some mannose/GlcNAc-binding proteins from chicken

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Chicken serum MBP</th>
<th>Chicken egg yolk MBP</th>
<th>Chicken liver GNBP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular mass, kDa</td>
<td>169 ± 7</td>
<td>169 ± 7</td>
<td>210*</td>
</tr>
<tr>
<td>Molecular structure, kDa</td>
<td>2 × 74; 2 × 27</td>
<td>2 × 74; 2 × 27</td>
<td>n × 27†</td>
</tr>
<tr>
<td>Immunochromatographical properties</td>
<td>Same as yolk MBP</td>
<td>Same as serum MBP</td>
<td>Different from serum/yolk MBP</td>
</tr>
<tr>
<td>Ca requirement</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Optimal pH for binding</td>
<td>5-9</td>
<td>5-9</td>
<td>7-9</td>
</tr>
<tr>
<td>Sugar specificity</td>
<td>Man = Fuc &gt; GlcNAc</td>
<td>Man = Fuc &gt; GlcNAc</td>
<td>GlcNAc &gt; Man = Fuc‡</td>
</tr>
<tr>
<td>Specific activity</td>
<td>408</td>
<td>342</td>
<td>250†</td>
</tr>
<tr>
<td>Amino acid composition</td>
<td>Similar to yolk MBP</td>
<td>Similar to serum MBP</td>
<td>Different from serum/yolk MBP‡</td>
</tr>
</tbody>
</table>

*MBP, mannose-binding protein; GNBP, GlcNAc-binding protein; Man, mannose; Fuc, L-fucose.

†Ref. 2.
‡Ref. 3.
§Ref. 24.

Expressed as ng of ¹²⁵I-labeled Man₃3-AI-BSA bound per µg of binding protein. For assay of the MBPs and GNBP, 100 nM and 580 nM ¹²⁵I-labeled Man₃3-AI-BSA were used, respectively.
Table 4. Animal mannos-binding proteins

<table>
<thead>
<tr>
<th>Source</th>
<th>Calcium required</th>
<th>Molecular mass, kDa</th>
<th>Binding hierarchy</th>
<th>Source or refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammalian (rat, rabbit, human)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung macrophage</td>
<td>+</td>
<td>175</td>
<td>Man &gt; l-Fuc &gt; GlcNAc</td>
<td>32, 33</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parenchymal cells</td>
<td>+</td>
<td>190</td>
<td>Man = GlcNAc &gt;&gt; Gal</td>
<td>34, 35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>32</td>
<td>ManNAc = Man = GlcNAc</td>
<td>36-40</td>
</tr>
<tr>
<td>Lymphoid tissue</td>
<td>+</td>
<td>32, 58</td>
<td>ManNAc = GlcNAc = Man = ManNH₂</td>
<td>41</td>
</tr>
<tr>
<td>Serum</td>
<td>+</td>
<td>60</td>
<td>ManNAc = GlcNAc = Man</td>
<td>6, 7</td>
</tr>
<tr>
<td>Avian (chicken)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>210</td>
<td>27</td>
<td>GlcNAc &gt; Man = l-Fuc</td>
<td>2, 4</td>
</tr>
<tr>
<td></td>
<td>280-740</td>
<td>32, 41, 70</td>
<td>ManNAc = GlcNAc = Man = l-Fuc</td>
<td>5</td>
</tr>
<tr>
<td>Serum</td>
<td>169</td>
<td>27, 74</td>
<td>Man = l-Fuc &gt; GlcNAc &gt;&gt; ManNAc</td>
<td>1; this work</td>
</tr>
<tr>
<td>Yolk</td>
<td>169</td>
<td>27, 74</td>
<td>Man = l-Fuc &gt; GlcNAc &gt; ManNAc</td>
<td>1; this work</td>
</tr>
</tbody>
</table>

Man, mannos; l-Fuc, l-fucose; Gal, galactose.

Table 4 compares the sugar-binding preferences of the known animal mannos-binding proteins. The preferences of the avian serum- and yolk-binding proteins are similar to the mammalian alveolar macrophage-binding protein and quite different from the others that bind GlcNAc better or comparable to mannos. In addition, the avian serum- and yolk-binding proteins poorly bind ManNAc, in contrast to the mammalian liver parenchymal cell, lymphoid tissue, mammalian serum, and one of the chicken liver mannos-binding proteins that bind ManNAc very well. The chicken serum and yolk mannos-binding proteins are unique among the mannos-binding proteins in that they do not require calcium for binding.

It is probable that mannos-binding antibodies are represented in each antibody class in the chicken, and the presence of mannos-binding immunoglobulins of only the IgG class in the yolk may be explained by its specific transport into the yolk. Why the chicken possesses a family of mannos-binding antibodies and the mechanism by which they function within the animal are questions of interest.

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