A Ca^{2+} -Dependent Phosphorylcholine-Binding Protein in Chicken Serum

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ABSTRACT. A Ca^{2+} -dependent phosphorylcholine (PC)-binding protein in normal chicken serum was purified by affinity chromatography on *p*-aminophenyl PC-Sepharose 4B followed by gel filtration on Sephacryl S-300. In gel filtration, the isolated PC-binding protein was eluted in a symmetrical protein peak at the position of approximately 100,000. On sodium dodecyl sulfate-polyacrylamide gel electrophoresis, the protein was resolved into two protein bands of 31,000 and 38,000 under nonreducing conditions and of 40,000 and 46,000 under reducing conditions. These results suggest that chicken serum PC-binding protein may be composed of two different subunits which contain intrachain disulfide bonds.—**KEY WORDS:** chicken serum protein, isolation, phosphorylcholine-binding.

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Human and animal sera contain carbohydrate-binding proteins in addition to natural antibodies which react with carbohydrates [6, 7, 10, 15, 17–21, 23]. Some of them are known as acute-phase reactants (or proteins) such as C-reactive protein (CRP), serum amyloid-P component (SAP), mannose and/or mannan-binding protein (MBP), and conglutinin (Kg) [4, 8, 9, 15, 17, 23]. The serum levels of CRP and SAP rapidly increase in human and mouse with acute inflammation due to infection or tissue injury [15–17]. Those acute-phase reactants are thus usually usable as clinical index [15]. Although isolation and characterization of bovine and canine CRPs have recently been reported [1, 5, 13, 14, 24], little is known about avian CRP [17]. Attempts were therefore made to purify Ca^{2+} -dependent phosphorylcholine (PC)-binding protein in chicken serum to identify and isolate chicken CRP.

Chicken sera were obtained from adult normal inbred chickens (line V: genotype B¹⁵B¹⁵) which had been maintained at the National Institute of Animal Health, Tsukuba, Japan. SeaKem HE agarose, *p*-aminophenyl PC-Sepharose 4B, CNBr-Sepharose 4B, N-acetylglucosamine-Sepharose 4B, and Sephacryl S-300 HR were the products of FMC Co., Ltd., Rockland, ME, U.S.A., Pierce Chemical Co., Rockford, IL, U.S.A. and Pharmacia, Uppsala, Sweden, respectively. Molecular markers for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were the products of Japan Bio-Rad Laboratories. Some of molecular markers for gel filtration were prepared in this laboratory according to the methods described previously [3, 22]. Bovine serum albumin and ovalbumin were the products of Sigma Chemical Company, St. Louis, MO, U.S.A.

Chicken IgM and IgG were isolated by affinity chromatography on N-acetylglucosamine-Sepharose 4B followed by gel filtration on Sephacryl S-300 as described previously [22]. Antiserum against chicken IgM and IgG was prepared in a rabbit by the methods described previously [21]. According to the methods of Cuatrecasas [2], rabbit anti-chicken IgM and IgG antibodies were isolated by affinity chromatography on chicken IgM and IgG-coupled Sepharose 4B and affinity-purified anti-chicken IgM and IgG antibodies were coupled to CNBr-Sepharose 4B.

For the isolation of Ca^{2+} -dependent chicken serum PC-binding protein, approximately 200 ml of pooled normal chicken sera was dialyzed against distilled water. The insoluble substances formed during dialysis were removed by centrifugation at $25,000 \times g$ at 4°C for 20 min. The supernatant was dialyzed against 10 mM Tris-HCl, pH 8.0, containing 0.2 M NaCl and 2 mM CaCl_2 (Buffer I). After the dialysis, the clear supernatant was taken by centrifugation at $25,000 \times g$ at 4°C for 20 min. The supernatant was subjected to the 4% SeaKem HE agarose column (2.5×20 cm) equilibrated with Buffer I as described previously [22]. The unadsorbed serum proteins were then subjected to the *p*-aminophenyl PC-Sepharose 4B column (1×5 cm) equilibrated with Buffer I. After washing the column with Buffer I extensively, adsorbed serum proteins were eluted with 10 mM EDTA in 10 mM Tris-HCl, pH 8.0, containing 0.2 M NaCl (Buffer II). After recalcification, the eluate was subjected to the same affinity column equilibrated with Buffer I. Adsorbed proteins were eluted with 10 mM PC in Buffer I. The eluate was concentrated by ultrafiltration in Amicon cell (Amicon Co., MA, U.S.A.) with a diaflo PM-30 membrane. Chicken immunoglobulins (mainly IgG) in the concentrate were absorbed with rabbit anti-chicken immunoglobulins-coupled Sepharose 4B. After the absorption, Ca^{2+} -dependent PC-binding protein(s) was again subjected to another *p*-aminophenyl PC-Sepharose 4B column (1×5 cm) equilibrated with Buffer I. Adsorbed PC-binding protein(s) was eluted with 5 mM PC in Buffer I. The eluate was concentrated by ultrafiltration. The concentrate was further purified by gel filtration on a Sephacryl S-300 HR column (1.5×90 cm) equilibrated with Buffer II or analyzed by gel permeation chromatography using two connecting Protein PAK 300 columns ($7.8 \text{ mm} \times 30 \text{ cm} \times 2$) (Nihon Waters, Tokyo, Japan) with a Waters HPLC system at a flow rate of 1 ml/min using 10 mM sodium phosphate buffer, pH 7.0, containing 0.2 M NaCl and 2 mM EDTA as an eluant.

SDS-PAGE was carried out by the methods described previously [11]. Protein content was determined by the methods of Lowry *et al.* [12] using bovine serum albumin as the standard. Immunogel double diffusion and preparation of rabbit anti-chicken PC-binding protein were carried out by the methods described previously [21].

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To isolate Ca^{2+} -dependent PC-binding protein in pooled normal chicken serum, Ca^{2+} -dependent agarose-binding proteins and Ca^{2+} -independent PC-binding proteins were removed by affinity chromatographies on both the SeaKem HE agarose and *p*-aminophenyl PC-Sepharose 4B columns. After the further removal of Ca^{2+} -dependent PC-binding immunoglobulins, Ca^{2+} -dependent PC-binding protein was purified by affinity chromatography on *p*-aminophenyl PC-Sepharose 4B followed by gel filtration on a Sephacryl S-300 column. In gel filtration of the PC-binding protein, a single protein peak appeared at the position of approximately 100,000 according to a calibration curve derived from different standards of known molecular weights. Approximately 0.25 to 0.5 mg of the PC-binding protein was purified from 200 ml of pooled normal chicken serum. In immunogel double diffusion with rabbit antiserum against chicken PC-binding protein, the purified PC-binding protein formed a single precipitin line, whereas chicken IgM and IgG did not (data not shown). Purified PC-binding protein was resolved into two protein bands with molecular weights of 31,000 and 38,000 in SDS-PAGE under nonreducing conditions and of 40,000 and 46,000 under reducing conditions (Fig. 1). These findings suggest that the PC-binding protein may be composed of two different subunits which contain intrachain disulfide bonds as found in the case with canine CRP [1, 5, 24] although chicken

Fig. 1. SDS-polyacrylamide slab gel electrophoresis using 10% gel. M, molecular markers; 1, unreduced purified chicken serum phosphorylcholine-binding protein; 2, reduced purified chicken serum phosphorylcholine-binding protein. Markers used were phosphorylase B (92,500), bovine serum albumin (66,200), ovalbumin (45,000), carbonic anhydrase (31,000), soybean trypsin inhibitor (21,500), and egg white lysozyme (14,400).

CRP has been demonstrated to be composed of a single subunit with a molecular weight of 21,000 [17]. The discrepancy in the molecular weights may be due to the difference in chicken strain. Because similar findings have been reported in canine CRP [1, 5, 24]. With respect to the subunits of canine CRP, their molecular weights from beagle dogs have been shown to be 85,000 and 70,000 [24] by the analysis on SDS-PAGE and those from mongrel, collie, and greyhound dogs to be 25,000 and 21,000 [1, 5].

The present findings suggest that the purified PC-binding protein may be a candidate for chicken CRP. More detailed studies on Ca^{2+} -dependent PC-binding protein in chicken serum will be needed to fully understand the role(s) of chicken CRP.

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