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Isolation and purification of riboflavin binding protein from ostrich egg (*Struthio camelus*)

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ABSTRACT

Riboflavin binding protein (RfBP) is one of the several nutrient-binding proteins with primary function of depositing riboflavin in the egg. Riboflavin binding protein was isolated and purified from Ostrich (Struthio camelus) egg white and yolk by DEAE Sepharose ion exchange chromatography followed by gel filtration chromatography on Sephadex G-100. The purity of the protein was judged by SDS-PAGE technique. Comparison of the mobility of the purified proteins with the standard molecular weight marker proteins revealed that the Ostrich RfBP (egg white and yolk) had a molecular weight close to 54 kDa and it was approximately 25 kDa larger than the hen egg white RfBP.

Key words: Riboflavin binding protein, Isolation of proteins, Ostrich egg.

INTRODUCTION

Ostrich (Struthio camelus) is a large flightless bird native to Africa, belonging to the ratite family. It is the largest living species of bird and lays the largest eggs of any living bird. Riboflavin-binding protein (RfBP) is one of the several nutrient-binding proteins found in eggs of birds and other vertebrates [1]. The RfBP of egg yolk, like other yolk proteins, is synthesized in the liver and transported by the blood stream to the developing oocyte [2]. The primary function of RfBP is to deposit riboflavin in the egg. The absence of RfBP from the blood and eggs of a mutant strain of chickens leads to the death of the developing embryo by reason of a gross riboflavin deficiency [3,4]. Thus, RfBP is shown to be responsible for the transport of riboflavin through the blood stream of the laying hen and into the eggs, where the vitamin is essential for embryonic growth and development [2,5]. Riboflavin carrier protein (RCP) from reptilian [6], Hen (*Gallus gallus*) and Coot Egg-Yolk (*Fulica atra)* [7], Parrot [8], amphibian [9], fish [10], eggs of Indian python, painted turtle [11], alligator [12], goose [13], Japanese quail [14], duck [15], peacock [16] and Emu [17] have been purified and characterised [18]. In the present study RfBP was purified for the first time from a single egg of ostrich (Struthio camelus). The isolated protein was characterized and compared with hen (Gallus gallus) egg white RfBP.

MATERIALS AND METHODS

Struthio camelus (Ostrich) egg was obtained from Private farms, at Bangalore, Karnataka. The whites and yolk were separated and used immediately or stored at -120^{o}C. DEAE- Sepharose was obtained from Sigma Aldrich Chemical Company, St. Louis, USA; Sephadex G-100 and Freund's Ceomplete adjuant from Sigma Aldrich Chemical Company. St. Louis, USA. Bovine Serum albumin, acrylamide, N, N, N1, N1- Tetramethylethylene- diamine, N, N1-methylene-bis-acrylamide and SDS were procured from Loba Chemical, Bombay, India. All other reagents were of analytical grade.

Isolation and purification of Ostrich egg white and yolk riboflavin binding protein (RfBP):
RfBP from Ostrich egg white and yolk was isolated following the methods previously reported [4,19-21] with a few modifications. Ostrich egg white albumin (1 egg, 750 ml) and yolk (1egg, 320 ml) was collected and homogenized with an equal volume of 0.1 M sodium acetate buffer pH 4.5. To the clear supernatant DEAE- Sepharose previously equilibrated with 0.1 M Sodium acetate buffer pH 4.5 was added. The DEAE- Sepharose with bound protein was washed with an excess of 0.1 M sodium acetate buffer pH 4.5. Bound proteins were eluted with the same buffer containing 0.5 M sodium chloride by suction filtration. Fresh DEAE Sepharose previously equilibrated with 0.1M sodium acetate buffer pH 4.5 was packed into the column and then the partially purified RfBP was loaded onto the column. Riboflavin binding protein was eluted from the column with 0.1 M sodium acetate buffer, pH 4.5 containing 0.5 M sodium chloride. Further purification of ostrich egg white and yolk RfBP was achieved by gel filtration column chromatography using Sephadex G-100. The almost pure Ostrich egg white and yolk RfBP was loaded onto the column previously equilibrated with 0.02 M phosphate buffer pH 7.3 containing 0.5 M sodium chloride and eluted with the same buffer. Fractions were collected and the protein in each fraction was determined by the method of Lowry *et al*., (1951) [22].

SDS-PAGE: Cylindrical Gel:
The gels were prepared by mixing 2 ml distilled water 8 ml running buffer, 4 ml acrylamide bisacrylamide solution, 20 TEMED AND 2 ml ammonium persulphate solution. The samples were dissolved in 50 of sample buffer. The samples were heated for 2 min. in a boiling water bath. 20 µl of the sample was loaded onto the gel tubes. The electrophoresis was carried out at 2-5mA/tube until the dye reached the end of the tube. The gels were staining overnight with distaining solutions, and stored in distilled water till they were photographed.

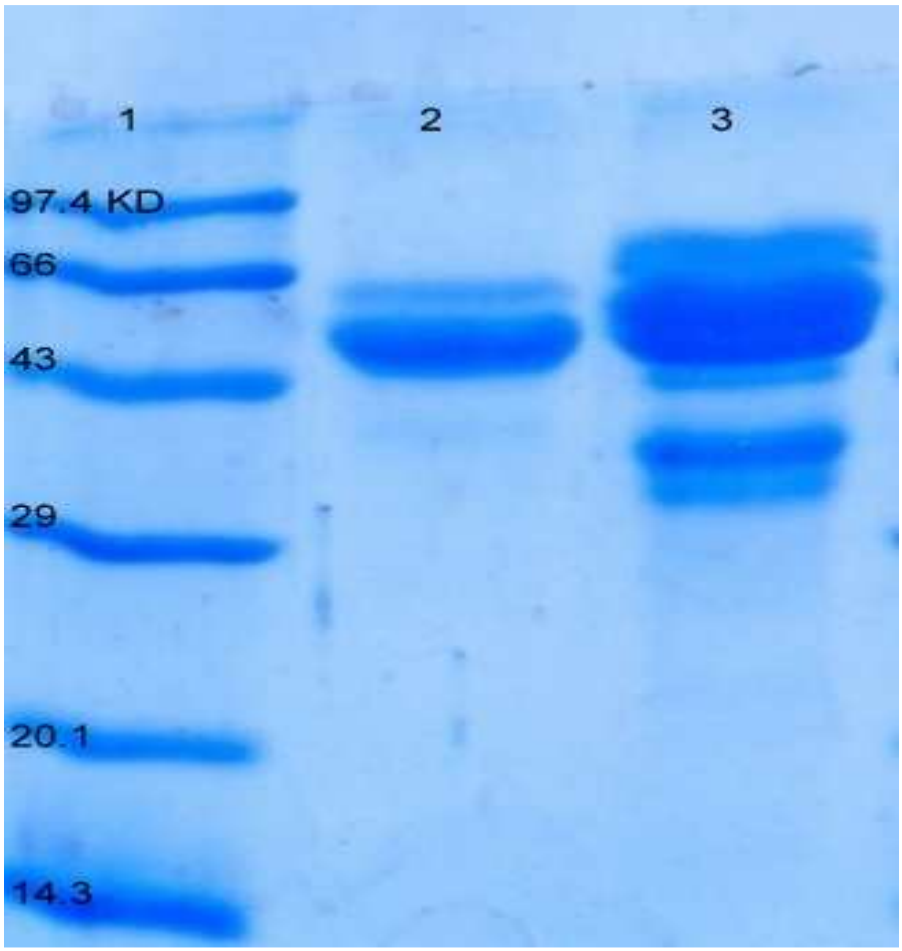
SDS-PAGE: Slab Gel:
The gel were prepared by mixing 4 ml of distilled water,16 ml electrode buffer, 8 ml acrylamide bisacrylamide, 40 µl TEMED and 4 ml ammonium per sulphate. The prepared gel solution was poured into glass plates (14x14 cm) separated by 1 mm thick spacer. The samples were dissolved in 50 µl sample buffer and kept in a boiling water bath for 2min. The ostrich egg white and yolk samples (20µl) were loaded into the slots. The remaining gap was filled with the electrode buffer. The glass plates were fixed to the electrophoresis apparatus without disturbing the samples. The upper and the lower electrode chamber were filled with the electrode buffer. The electrode chambers were connected to the power supply. Initially electrophoresis was carried out at 15mA for 30 min, after which the current was raised to 30mA. Current supply was terminated when the tracking dye reached the end of the gel. The plates were removed from the chambers, the gel was removed from the glass moulds by flushing buffer between the plates. The gel was stained immediately at room temperature. Later the gels were destained using the destaining solution.

RESULTS AND DISCUSSION

In the present study, crude egg white and yolk fractions from ostrich and hen egg white were prepared as described in the materials and methods section. The clear yellow supernatant fractions obtained after centrifugation were used for batch adsorption onto DEAE Sepharose. The bound proteins were eluted with 0.1M Sodium acetate buffer pH 4.5 containing 0.5M NaCl after thoroughly washing the DEAE Sepharose to remove the nonspecific proteins with 0.1M Sod acetate buffer pH 4.5. The eluted proteins were dialyzed against 0.1M Sodium acetate buffer pH 4.5, and loaded on a fresh DEAE Sepharose column and the bound RfBP was eluted as described earlier. The fractions having highest absorbance were pooled and used for further purification. A visible absorption spectra revealed that the RfBP had a characteristic of riboflavin apo protein complex (holo protein). The absorption peak of ostrich egg whiteand yolk shows the remarkable hypochromism, which was exactly the similar spectral data reported for hen egg white RfBP [23, 24].

The purity of the isolated RfBP were judged by native and SDS PAGE (slab). The results were shown in fig 1. A nearly homogenous major RfBP band with a few minor bands corresponding to other impurities could be seen after the 2 step ion-exchange binding with DEAE Sepharose. The SDS PAGE analysis revealed that the ostrich egg white and yolk RFBP had a molecular weight of approximately 54KDa, fig 1. Comparison of the molecular weights of the RfBPs from ostrich and hen revealed that the RfBP from the ostrich egg white and yolk had a molecular weight of approximately 25KDa larger than the hen egg white RfBP. Earlier it was reported that the RfBP from emu egg white had approximately 10KDa larger than hen egg white RfBP. Thus, the ostrich egg RfBP appeared to be having highest molecular weight recorded in the existing avian species. It is presumed that the large difference in the molecular weights (ratite) could be attributed to the differences in the glycosylation patterns [25].

Fig 1: SDS-Polyacrylamide Gel Electrophoresis Pattern of Ostrich egg RfBP on SLAB gels



1. Protein Marker; 2. Ostrich egg white; 3. Ostrich egg yolk.

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