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GLYCOPROTEIN ESTIMATION OF RIBOFLAVIN BINDING PROTEIN (SILVER STAINING) FROM THE EGGS OF COOT (*Fulica atra)* AND HEN (*Gallus gallus*): A COMPARATIVE STUDY

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ABSTRACT:

Riboflavin binding protein (RfBP) was isolated from the eggs of *Fulica atra* and *Gallus gallus.* The protein was purified in two steps, DEAE- Sepharose ion exchange chromatography followed by gelfilteration on Sephadex G-100. The purity of the protein was judged by SDS-PAGE technique. The Glycoprotein Estimation of Riboflavin binding protein. A single band on the slab and cylindrical gels revealed that the protein was pure. Comparison of the mobility of RfBP with that of the standard molecular weight marker proteins suggested that RfBP from the egg white and yolk of *Fulica atra and Hen* had a molecular weight close to 29 Kd.

KEY WORDS: Riboflavin binding protein (RfBP), DEAE-Sepharose, Electrphoretic characterization, Molecular weight Silver staining kit.

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INTRODUCTION:

Riboflavin binding protein (RfBP) is a Phosphoglycoprotein RfBP was first isolated from the chicken egg white.[1]. RfBP a molecular weight of 29.100 containing 219 Amino acid residues[2]. The isolation of RfBP from egg yolk was first published[3] and improved methods were subsequently reported[4]. Riboflavin binding protein which ensures deposition of adequate amount of the vitamins in the avin eggs was indispensable for a normal hatch of the fertilized eggs. Inadequate deposition of the vitamin in the eggs due tosplice mutation of riboflavin binding protein gene led to the embryonic mortality[5]. In addition the demonstration that immune-neutralization of riboflavin binding

protein result in the abrupt termination of pregnancy in animals such as rates and monkeys[6]. Those methods slightly modified in the isolation of RfBP from Eagle egg white and yolk[7]. In the present study RfBP was purified for the first time from coot and Hen egg white and yolk RfBPs glycoprotein Estimation (Silver staining) for a comparative study in terms of gross molecular characteristics such as molecular weight similar.

MATERIALS & METHODS:

Fulica atra eggs were collected from Nagaram Lake which is located in Warangal district, Andhra Pradesh, and Hen eggs were procured from local shayampet Hanamkonda. The white and yolk were separated and used immediately or stored at -12^{0}C. DEAE-Sepharose and Sephadex G-100 were obtained from Sigma Aldrich Chemical Company. St. Louis, USA. Silver staining kit (Bangalore Genei Ltd) ,Bovine Serum albumin, acrylamide, N, N, N^1, N^1-Tetramethylethylene- diamine, N, N^1-methylene-bis-acrylamide and SDS were procured from Loba Chemical, Bombay, India.

Isolation and purification of riboflavin binding protein (RfBP):

RfBPs from *Coot and Hen* egg white and yolk were isolated following the methods previously reported with a few modifications. *Coot and Hen* egg white or yolk 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, to remove the unbound proteins. 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. Fractions were collected and absorbances were measured at 280 nm for proteins and 455nm for bound riboflavin. Further purification of Coot and Hen egg white and yolk RfBPs were achieved by gel filtration column chromatography using Sephadex G-100. The almost pure Coot egg white RfBP was loaded onto the column previously equilibrated with 0.02 M phosphate buffer pH 7.3 containing 0.5 M sodium chloride and immediately eluted with the same buffer. Fractions were collected and the protein in each fraction was determined by the method of Lowry[8].

SDS-PAGE on cylindrical and slab gels were carried out as described earlier[9,10] following the method of Leammli[11].

SDS– PAGE Silver Staining:

The proteins were also stained by the silver staining method to obtain a more sensitive image of the protein bands. A Silver staining kit (Bangalore Genei Ltd) was used and the following reagents were prepared:

Reagent preparation:

1) Fixing solution: 25ml of the given fixing solution was made up to 50ml with distilled water.

2) Sensitizing solution: 14 ml of 25% Glutaraldehyde, 1ml of 10% sodium thiosulphate & 11.6ml of 10% sodium acetate were made up to 50ml with distilled water.

3) Silver solution: 620µl of 20% Silver Nitrate and 20µl 37% formaldehyde were made upto 50ml with distilled water.

4) Developing solution: 12.6ml of sodium carbonate and 20µl of 37% formaldehyde were mixed and made upto 50 ml.

5) Stop solution: 5ml of the stop solution was made up to 50 ml with distilled water.

RESULTS & DISCUSSION:

The electrophoretic pattern obtained on the cylindrical gels (Fig .1) and slab gels (Fig.2) using purified Coot and Hen egg white &yolk RfBP along with protein molecular weight markers revealed that the RfBPs from both the bird eggs had a molecular weight close to 29 kDa. Similarly ,the mobilities of RfBPs isolated from Coot and Hen egg white and egg yolk on cylindrical (Fig.3) clearly indicated that these RfBPs also had a molecular weight close to 29kDa. Further, silver staining(Glycoprotein Estimation) of purified RfBPs from Coot egg white & yolk as well as Hen egg white &yolk after SDS-PAGE on slab gels established that these RfBPs had similar electrophoretic mobilityes having the same molecular weight of 29kDa.

The present study clearly showed that the RfBP isolated from egg white and egg yolk of the flying & water born bird, Fulica atra (Coot) had an electrophoretic mobility similar to that of the RfBP isolated from the non flying bird ,the Hen egg white and egg yolk , having a molecular weight close to 29kDa.

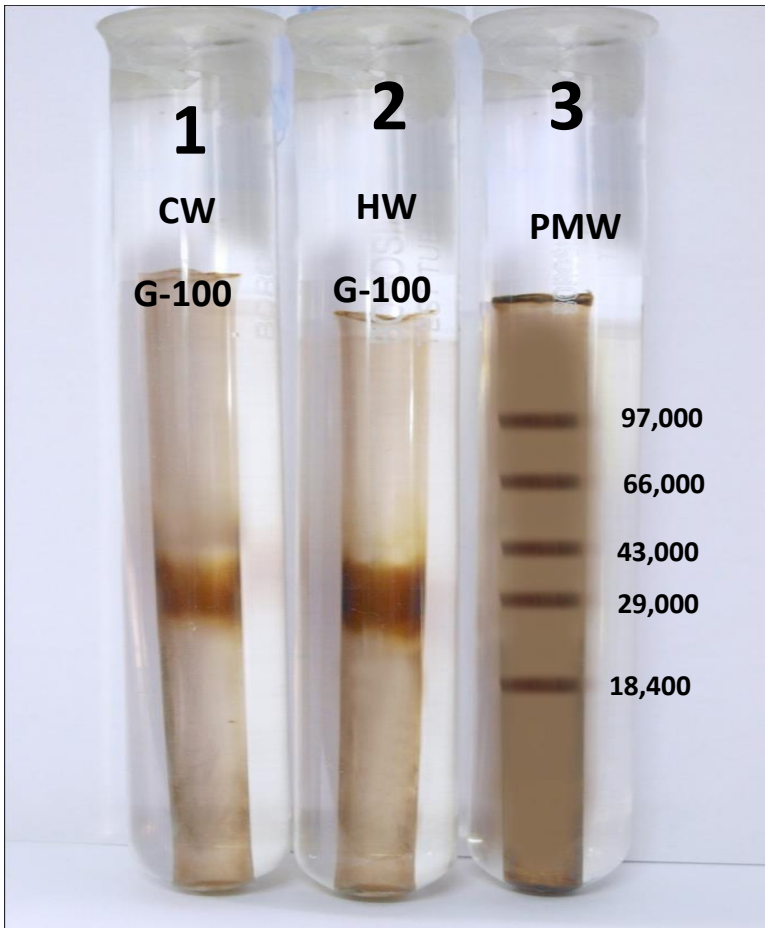

Fig : 1 Silver staining of Cylindrical Gel Electrophoresis (SDS Polyacrylamide) pattern of Coot egg white and Hen egg white RfBP's

1. Coot egg white G-100 eluted fraction
2. Hen egg white G-100 eluted fraction
3. Protein molecular weight marker (18,400 to 97,000 kD

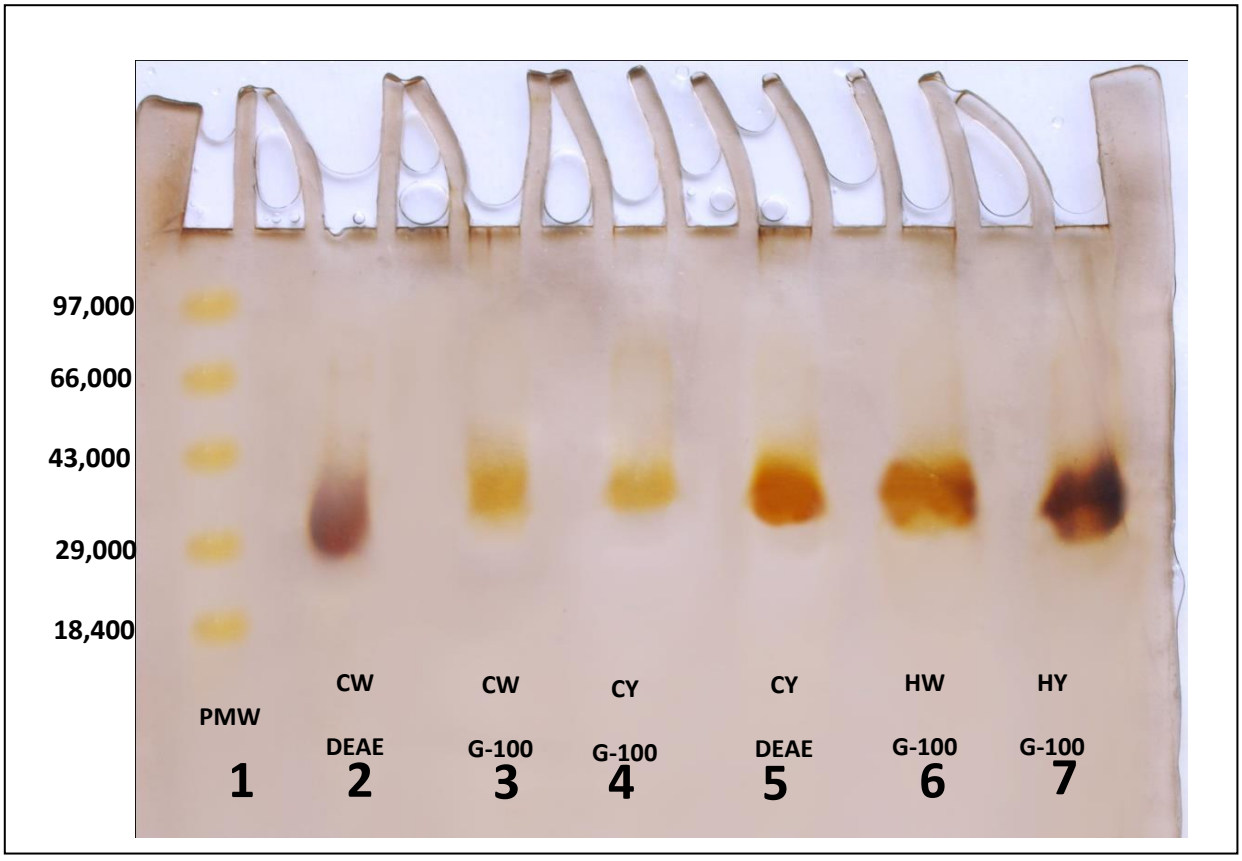

Fig : 2 Silver staining on SDS Polyacrylamide Gel Electrophoresis pattern of Coot egg white and egg yolk RfBP's

1. Protein molecular weight marker (18,400 to 97,000 kD)
2. Coot egg white DEAE Sepharose eluted fraction
3. Coot egg white Sephadex G-100 fraction
4. Coot egg yolk Sephadex G-100 fraction
5. Coot egg yolk DEAE Sepharose eluted fraction
6. Hen egg white Sephadex G-100
7. Hen egg yolk Sephadex G-100

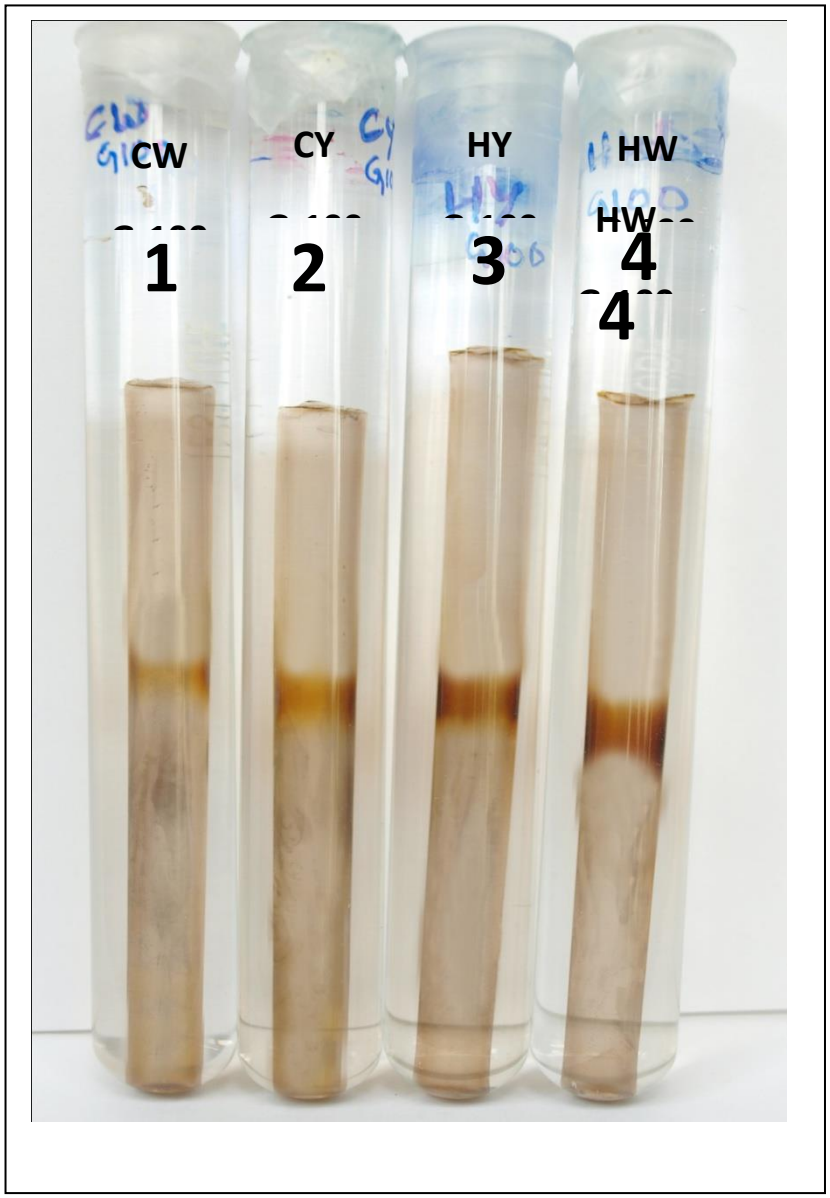

Fig : 3 Cylindrical Gel Electrophoresis (SDS Polyacrylamide) pattern of Coot egg white and Hen egg white RfBP's

1. Coot egg white G-100 eluted fraction
2. Coot egg yolk G-100 eluted fraction
3. Hen egg yolk G-100 eluted fraction
4. Hen egg white G-100 eluted fraction

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