Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2015; 7(1); 27-29

ISSN: 0975-4873

Research Article

Purification and Characterization of Riboflavin Binding Protein from the Egg White of Coot (*Fulica atra)*

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Available Online: 1st February, 2015

ABSTRACT
Riboflavin binding protein (RfBP) was isolated from the eggs of *Fulica atra*. The protein was purified in two steps, DEAE-Sepharose ion exchange chromatography and gelfilteration on Sephadex G-100. The holoprotein had an absorption spectrum characteristic of flavoproteins. The purity of the protein was judged by SDS-PAGE technique. 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 of *fulica atra* had a molecular weight close to 29 Kd.

Keywords: Riboflavin binding protein (RfBP), DEAE-Sepharose, Electrphoretic characterization, Molecular weight.

INTRODUCTION

Riboflavin is unique among the water soluble vitamins in that egg, milk and dairy products make the greatest contribution to its intake in Indian diets. Meat and fish are also good sources of riboflavin and certain fruits and vegetables especially dark-green vegetables contain reasonably high concentrations[1]. Biochemical signs of depletion arise within only a few days of dietary deprivation. Poor riboflavin status in western countries also seems to be of most concern for the elderly and adolescents. Riboflavin deficiency has been implicated as a risk factor for cancer, although this has not been satisfactorily established in humans.

Recent studies have established that vitamin binding proteins from a special group of soluble proteins present in eggs and other body fluids which ensure optimal bioavailability of the vitamins during growth and development. The indispensability of RfBP has demonstrated in a study on the homozygous recessive mutant (rd-rd) of domestic foul wherein, a gene mutation leading to the absence of RfBP resulted in the death of the developing embryos[2]. Further the discovery that immunoneutralization of RfBP in animals such as rats and monkeys resulted in the abrupt termination of pregnancy clearly established the functional significance and RfBP[3].

The present investigation was carried out to isolate and characterize RfBP from egg-white of *Fulica atra*. *Fulica atra* belongs to rail family Rallidae, characterized by typical dark plumage toes that are lobed with a membrane, a dark head with a short, wide beak that extends from the base to the forehead as a prominent flattened, fleshy frontal shield or other decorcation on the forehead. These birds have predominantly black plumage and unlike many of the rails, they are usually easy to see and locally known as *Budubunga.* Earlier, RfBP was first isolated from the chicken egg white[4] (Rhodes *et al.,* &1959) and recently from peacock (*Pavocristatus*) egg-white[5,6] (Rajender, 2007 and 2009). Thoese methods were slightly modified for the isolation of egg white RfBP from *Fulica atra*.

MATERIALS AND METHODS

Fulica atra eggs were procured from Mucharla Lake which is located in Warangal district, Andhra Pradesh. DEAE-Sepharose, Sephadex G-100 and Freund's complete adjuvant used in the present study were obtained from Sigma Chemical Company, St. Louis, USA. 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 Fulica atra egg white RfBP

RfBp from *Fulica atra* egg white (from 25 eggs) was isolated following the methods previously reported[4, 7, 8] (Rhodes et al., 1959; Farrell et al., 1969; Hamazume et al., 1984) with a few modifications.

Fulica atra egg-white 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 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

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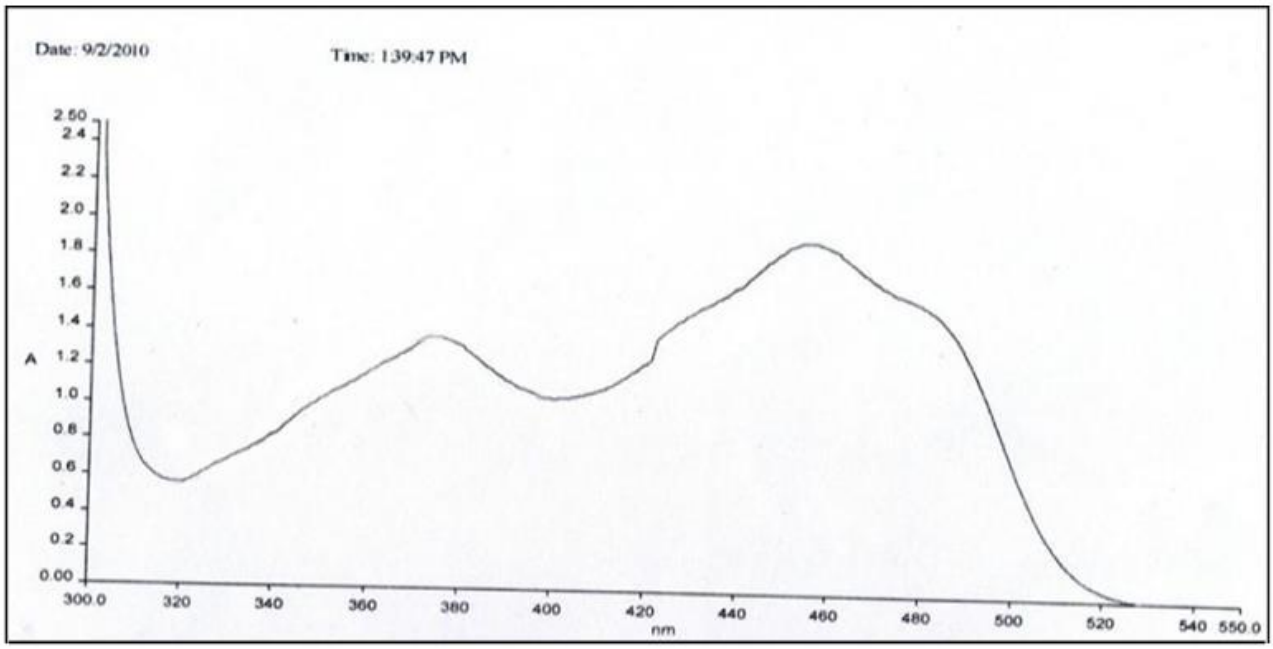

Fig 1: Absorption spectrum of *Fulica atra* egg-white RfBp (Sephadex G-100 Fraction)
[The peak fraction obtained after Sephadex G-100 gel filtration column chromatography was used to record the absorption spectrum using UV-Visible recording spectrophotometer. The holoprotein showed absorption maxima at 376 nm and 458 nm characteristic of riboflavin apoprotein form.]

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 and 455nm. Further purification of *Fulica atra* egg-white RfBP was achieved by gel filtration column chromatography using Sephadex G-100.

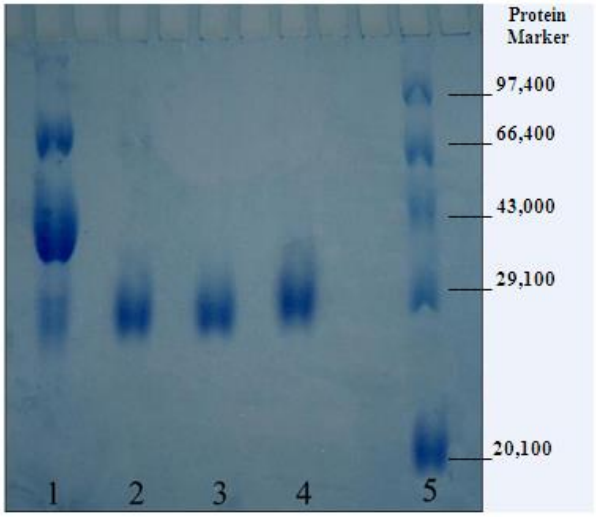

Fig 2A. SDS- PAGE pattern of *Fulica atra* egg-White RfBp, 1:RfBP Fraction (Crude), 2: RfBP Fraction (Batch eluted), 3: RfBP Fraction (DEAE-Sepharose eluted), 4: RfBP Fraction (Sephadex G-100), 5: Protein Molecular Weight Marker (20,000 to 97,400 Da)

[Complete purification was achieved by gel-filtration chromatography on Sephadex G-100, as a single band free from other minor contaminating proteins was obtained. RfBp moved as a single band on the SDS-PAGE. Comparison of the mobility of RfBp with that of the standard molecular weight marker proteins revealed that the RfBp had a molecular weight close to 29,000 Da.]

The column was equilibrated with 0.025 M phosphate buffer pH 7.3 containing 0.5 M sodium chloride. Protein in each fraction was determined by the method of Lowry et al., [9](1951).

Fulica atra egg-white RfBP samples were dissolved in 50µl sample buffer and kept in a boiling water bath for 2 minutes. Samples (20µl) were loaded into the slots of SDS-PAGE slab gels. Initially electrophoresis was carried out at 15mA for 30 minutes after which the current was raised up to 30 mA. After the electrophoresis, the plates were removed from the chamber and gel was detached by flushing distilled water between the plates. The gel was stained immediately at room temperature. Later the gel was destained using the destaining solution.

RESULTS AND DISCUSSION

The peak fraction obtained after Sephadex G-100 gel filtration column chromatography was used to record the absorption spectrum using UV-Visible recording spectrophotometer. As could be seen from Fig-1 the holoprotein showed absorption maxima at 376 nm and 458

nm characteristic of riboflavin apoprotein form[10] (Choi and Mc Cormic, 1980).

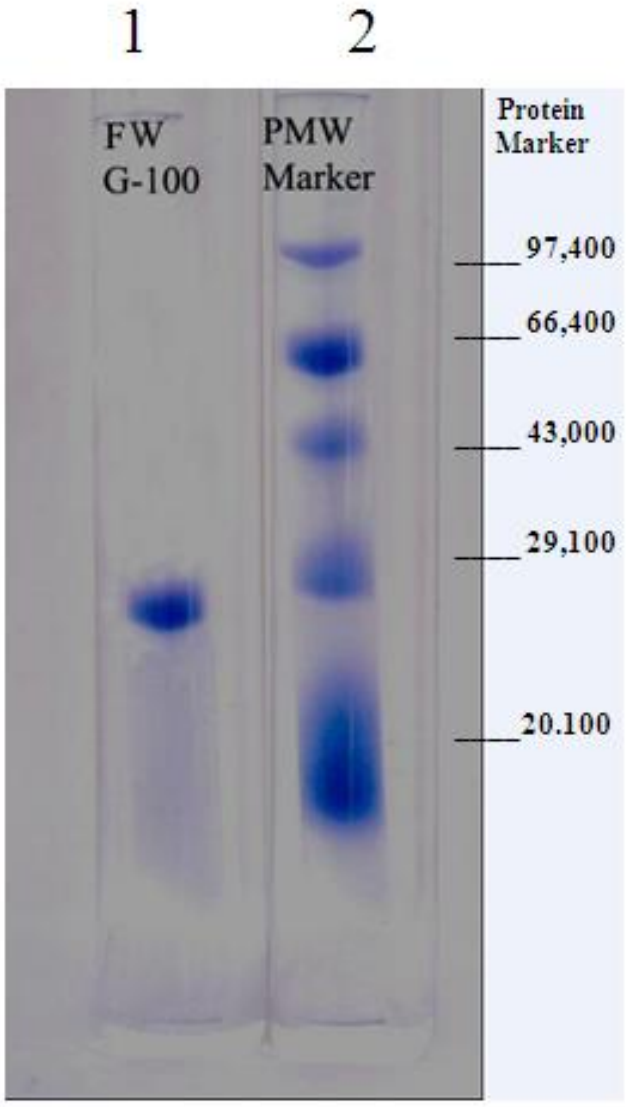

Fig-2B: Cylindrical gel SDS-PAGE pattern
Fulica atra egg-White., 1: *Fulica atra* egg-White Sephadex G-100 Fraction, 2: Protein Molecular Weight Marker (20,000 to 97,400 Da)

Electrophoresis on analytical polyacralamide gels (7.5%) was conducted at pH 8.3. The purity of the isolated protein was judged by slab and cylindrical SDS-PAGE methods. The electrophoretic pattern obtained was shown in Fig. 2. A major band corresponding to RfBp along with few minor bands were seen in the DEAE-Sepharose eluted fraction. Complete purification was achieved by gel-filtration chromatography on Sephadex G-100, as a single band free from other minor contaminating proteins was obtained (Fig. 2). RfBp moved as a single band on the cylindrical gels also (Fig. 2b). Comparison of the mobility of RfBp with that of the standard molecular weight marker proteins revealed that the RfBp had a molecular weight close to 29 kilodaltons.

Thus the present study clearly showed that RfBP from *Fulica atra* (flying bird) had an electrophoretic mobility similar to that of hen (non flying bird) egg-white RfBp having a molecular weight close to that the hen egg-white RfBp suggesting that this protein mostly remained unaltered in these species.

ACKNOWLEDGEMENT

Authors are thankful to Head, Department of Biochemistry and Chemistry for providing laboratory facility and also to the staff, Central Instrumentation Centre (CIC), Kakatiya University, Warangal, Telangana state.

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