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Research article

PURIFICATION AND CHARACTERIZATION OF RIBOFLAVIN CARRIER PROTEIN FROM EGG WHITE OF SOUTH INDIAN SPOTTED OWLET (*ATHENE BRAMA*)

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ABSTRACT : Riboflavin carrier protein has been isolated and purified from the Indian spotted owlet. The protein was purified to its homogeneity. Purification was achieved successfully by DEAE_ Sepharose column chromatography and gel filtration chromatography on Sephadex G-100. The protein content was estimated with Lowry method. The purity of the proteins was judged by SDS-PAGE technique. The molecular weight of the protein was found to be 29 Kd. The protein was characterized using absorption, fluorescence and CD spectral analysis. Significance of the above results are discussed in the present communication.

Key words: *Athene brama*- Egg-White, Riboflavin Carrier protein (RCP), purification.

INTRODUCTION

Spotted Owlet (*Athene brama*) is a small owl which is found in tropical Asia from India to Southeast Asia. It is a common resident of open habitats including farmland and human habitation and in the hollows of trees or rocks. Riboflavin is required in adequate amounts for normal fetal development; hence a specific carrier system of RCP has evolved for the developing embryo. Riboflavin carrier protein (RCP) from reptilian (Hamajima and Ono, 1995), Hen (*Gallus gallus*) and Coot Egg-Yolk (*Fulica atra)* (Madhukar Rao et.al.,2012) amphibian (Storey et al., 1999), and fish (Wang et al., 2003) eggs of Indian python, painted turtle (Abrams et al., 1988) alligator (Abrams et al., 1989) goose (Stevens et al., 1994), Japanese quail (Walker et al., 1991), duck (Muniyappa and Adiga, 1980), and peacock (Rajender et al., 2007) and Emu (Bindu et al., 2010) have been purified and characterised. Antibodies against chicken RCP caused termination of pregnancy in rats demonstrating the essential role of RfBP in the survival of the fetus (Krishnamurty *et al*., 1984), mice (Natraj *et al*., 1987) and the bonnet monkey (Visweswariah and Adiga, 1987). Increased levels of RBP were found in the serum of breast cancer patients and may be useful as a marker for breast cancer (Rao et al. (1999)). There are no reports of RfBP structure from egg white of Indian Spotted Owlet. In this paper, we discuss the structural elucidation and purification of RfBP from Indian Spotted Owlet.

MATERIALS AND METHODS

Indian Spotted Owl eggs were procured from, Old City, Hyderabad, and Andhra Pradesh. The white were used immediately or stored at -120C. DEAE- Sepharose, Sephadex G-100 and was obtained from Sigma Aldrich Chemical Company. St. Louis, USA. Riboflavin Carrier protein from hen egg-white and was isolated by the following methods of Farell *et al*. (1969) with a few modifications as described. Hen egg-white and yolk were collected and homogenized with an equal volume of 0.1 M sodium acetate buffer pH 4.5. The homogenate was processed with stirring over night. To the crude yellow supernatant, DEAE-Sepharose, previously equilibrated with 0.1 M sodium acetate buffer pH 4.5, was added and stirred overnight at 4ºC. The DEAE Sepharose was washed extensively with 0.1 M sodium acetate buffer; pH 4.5. The bound protein was eluted with same buffer containing 0.5 M NaCl by suction filtration. Eluted protein was loaded onto the DEAE-Sepharose column and washed with the 0.1 M sodium acetate buffer pH 4.5. The bound protein was eluted with same buffer containing 0.5 M NaCl**.** Twenty five fractions (5 ml each) were collected. SDS-PAGE was carried out according to the method of Leammli, (1979) using sodium phosphate buffer containing SDS.

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Protein content was estimated by the method of Lowry (1951). The gels were prepared by mixing 4ml distilled water, 16ml of electrode buffer, 8ml acryl amide bisacrylamide, 40µl TEMED and 4ml ammonium per sulphate solution. *Athene brama* Egg –White purified RCP samples were dissolved in 50µl sample buffer and kept in a boiling water bath for 2 minutes. Samples (20µl) were loaded into the slots. Initially electrophoresis was carried out at 15mA for 30 minutes after which the current was raised up to 30 mA. 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

Domestic fowl RCP which is the most abundant egg white binding protein has been most thoroughly investigated. It is a single polypeptide chain of 219 aminoacids with a molecular mass of 29 Kd. RfBP undergoes post–translational modifications, such as glycosylaton and phosphorylation as well as proteolysis. Egg-yolk RfBP is synthesized in the liver, and egg white RfBP in the oviduct. Rhodes *et al*. (1958) first reported the isolation and purification of RfBP from chicken egg white. In the present investigation RCP was purified by employing ion exchange chromatography on DEAE –Sepharose as one major band of RCP with minor contaminants which were separated using gel filtration on Sephadex G-100 to obtain a single band. The fractions were monitored for absorbance at 280 nm and 455 nm (fig.1). The peak fractions (6 Th to 11 Th) which were yellow in color with highest absorbance at 280 nm were pooled dialyzed against distilled water and lyophilized.

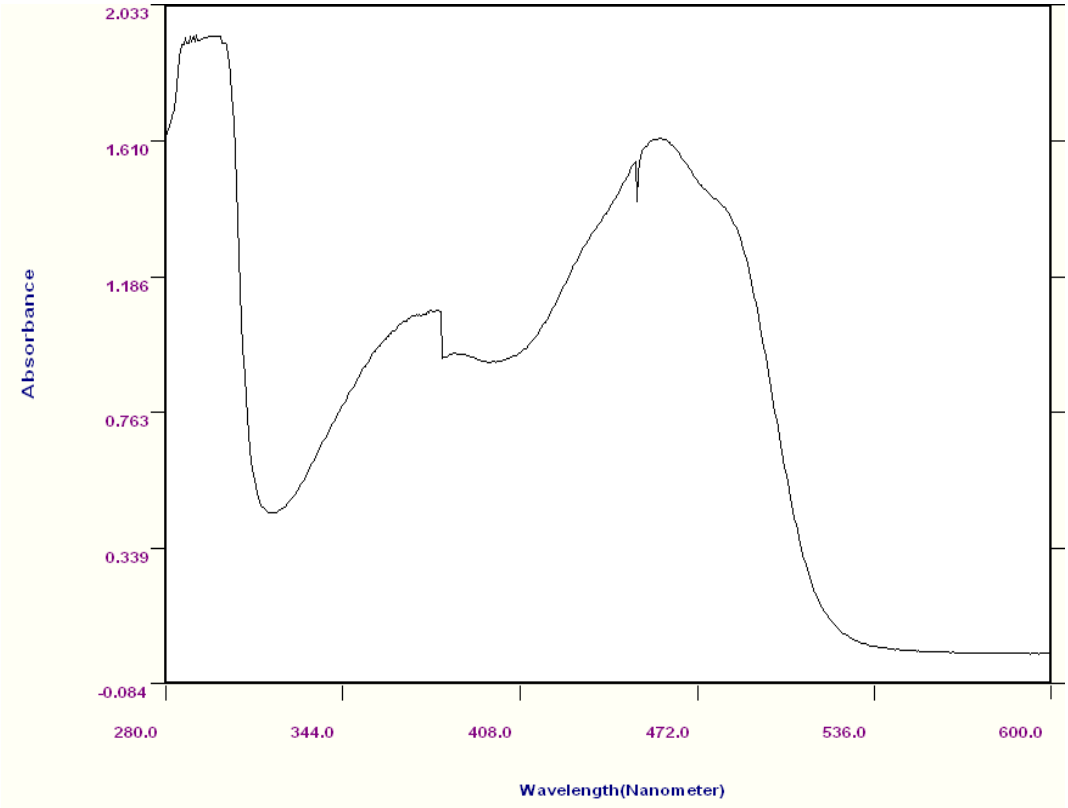

Figure – 1: Absorption Spectrum of *SPOTTED OWLET* Egg –White RfBP (Sephadex G-100 Fraction)

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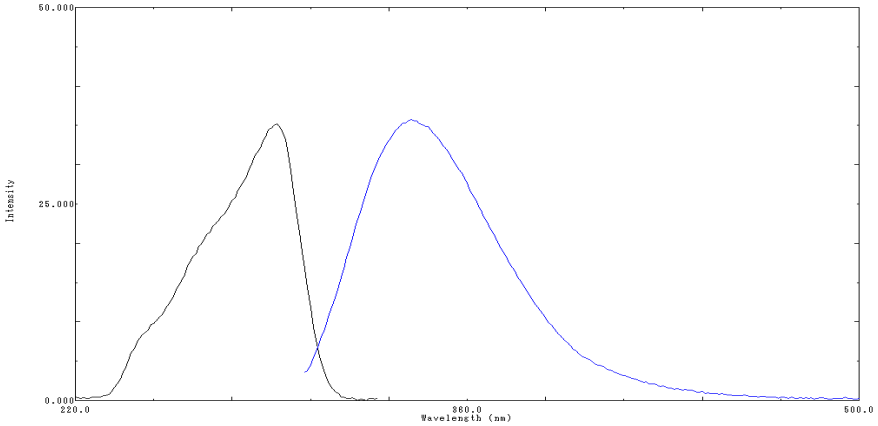

Figure-2: *Spotted owlet* Egg White Riboflavin Binding Protein Florocesence spectra

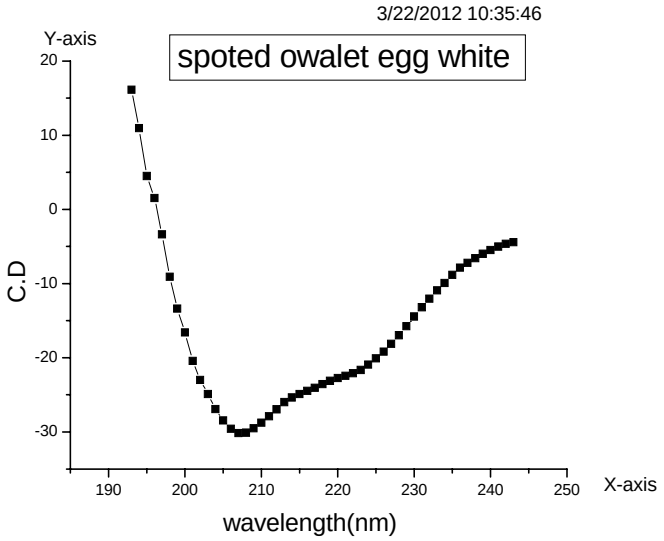

Figure -3: Far U.V.C.D Spectra of Riboflavin binding protein from spotted owlet egg white

Amino acid Analysis of spotted owlet Egg White RfBP (BeckHPLC Amino acid Analysis)

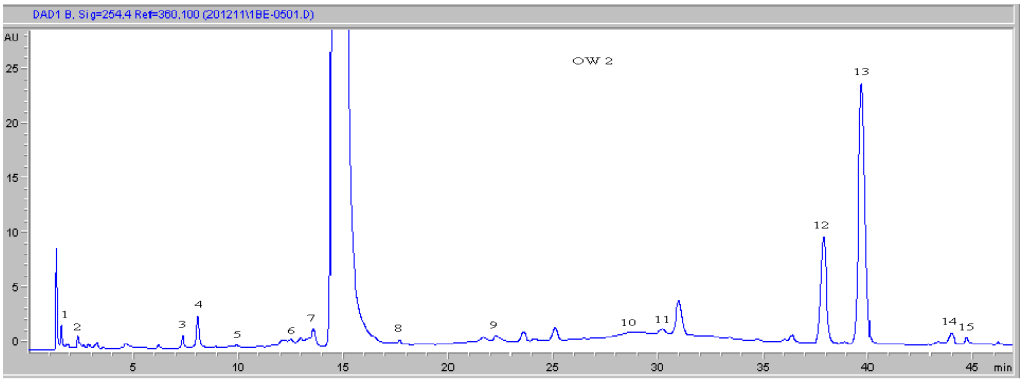

Figure-4: Spotted owlet egg white sample separation details and concentration by HPLC

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Table-1: Amino acid content of purified spotted owlet Egg White riboflavin binding protein

Sl.No.	Name of Amino acid	Sample Conc. (pmol)
1	P. Serine	0.007408053
2	Glutamic acid	0.006574483
3	Serine	0.008943992
4	Aspargine	0.033600566
5	Taurine	0.004762254
6	β-amino butyric acid	0.002444018
7	Histidine	0.01
8	Carnosine	0.00495886
9	Anserine	0.010305398
10	Tyrosine	0.022240234
11	Valine	0.008114882
12	Cysteine	0.177701763
13	Leucine	1.443082208
14	Phenylalanine	0.009377974
5	Ornithine	0.001446476

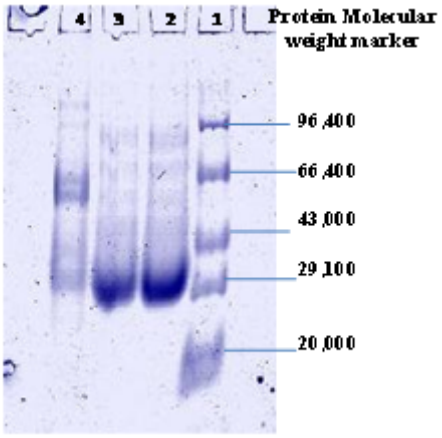

Fig: **SDS-PAGE Pattern of Spotted Owlet Egg White**

1. Protein Molecular Weight marker(20,000Da to 96,400)
2. Spotted owlet Egg White RfBP,DEAE-sephadex-100
3. Spotted owlet Egg White RfBP, DEAE- Sepharose Elution Fraction
4. Spotted owlet Egg White RfBP, DEAE- Sepharose Batch process

The protein with bound Riboflavin (holo protein) showed a typical spectrum similar to flavin-apo-protein complex. The spectral data confirmed that the purified protein was RfBP (Rhodes et, al., 1959), (Choi and Mc Cormick, 1980), (Rao et al., 2011). Far u.v.c.d spectrum (198 to 250 nm) of Owlet egg white RfBP is shown in fig.3. It has a sharp minimum at 206nm and a shoulder at 210nm. The near u.v.c.d of Owlet egg white RfBP fluorescence spectra recorded with excitation at 280nm and 295nm. They have a maximum at 340nm which reveals the presence of the protein. The far u.v.c.d. of hen egg white and owlet white RfBPs were shown to be similar (Nishikimi and Kyogoku, 1973).

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The amino acid analysis of the isolated pure Owlet egg white RfBP was analyzed on a Beckman HPLC amino acid analyser (fig 4). The amino acid composition of duck egg white RfBP was initially reported by (Muniyappa and Adiga, 1980). They found significant differences in the amino acid composition when compared with hen egg white RfBP. On the other hand they observed that the amino acid composition of goose egg-yolk and hen egg-yolk were quite similar. However they found significant differences in amino acid composition between the goose and duck yolk RfBPs particularly in proline, arginine, methionine, phenylalanine and histidine contents. The amino acid analysis of quail egg white RfBP revealed close similarities not only between quail egg yolk RfBP but also between quail RfBPs and Hen RfBPs. The amino acid analysis of the isolated Spotted Owlet egg white RfBP was analyzed on a Beckman HPLC amino acid analyser. The amino acid composition of Spotted Owlet egg white is shown in the Table 1. The electrophoretic pattern obtained is shown in fig 5 shows the molecular weight of the protein to be around 29,000 Da (approx). RCP from egg yolk of owlet showed a clear band confirming its homogeneity. Thus the protein could be purified by employing the above techniques. When compared to the molecular weight of hen egg yolk RCP, the molecular weight of the protein was similar. This is the first report of purification of RCP from Spotted Owlet.

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