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Purification and Characterization of Riboflavin Binding Protein (RfBP) in Egg-yolk of Peacock (*Pavo cristatus*)

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ABSTRACT

Riboflavin binding protein (RfBP) was isolated from the peacock (*Pavo cristatus*) egg-yolk. The protein was purified on DEAE-Sephadex A-50 ion exchange chromatography. The final purification of protein was on sephadex G-100. The purity of the protein was by cylindrical and slab –gels, SDS-PAGE technique. This protein showed a single band on SDS gels and the molecular weight was 30 Kilodaltons.

Keywords: Peacock (*Pavo cristatus*), Riboflavin, Purification and Characterization.

Introduction:

The specific binding proteins for fat soluble vitamins such as vitamin A and vitamin D are identified in the serum of the vertebrates [1-4].The binding protein for water soluble vitamins [5,6] Vitamin B_{12} [7,8] and Thiamin [9,10] have been demonstrated in the blood serum, egg-white and egg-yolk of the laying hens. The essential role of Riboflavin binding protein (RfBP) has been demonstrated in the homozygous recessive mutant (rd rd) domesting fowl [11] and in heterozygous leg horn hen [12, 13]. The Riboflavin binding protein (Rfbp) from peacock egg-white was purified and characterized [14].

The present investigation was undertaken with the aim of isolation, purification and characterization of riboflavin binding protein (RfBP) in egg-yolk of peacock (*Pavo cristatus*).

MATERIAL AND METHODS

The peacock eggs were collected from Gopalpur hillocks located 10 km away from Kakatiya University campus in Warangal district of A.P., which is a tropical region. The DEAE-Sephadex A-50 was obtained from Pharmacia Fine Chemicals, Uppasala, Sweden. Sephadex G-100 and Freund's complete adjuvant was procured from Sigma-Aldrich Chemical Company, St. Louis, U.S.A. The bovine serum albumin acrylamide N, N, N', N' -Tetramethylethylene diamine, N,N'-methylene-bis-acrylamide, Sodium dodcyal sulphate (SDS) were obtained from Loba Chemical Company, Bombay, India.

The riboflavin binding protein was isolated following the basic methods as described earlier [5, 12].Peacock egg-yolk riboflavin binding protein (RfBP) was purified to apparent homogeneity in two steps. Batch adsorption to DEAE-Sephadex and gel filtration column chromatography on Sephadex G-100 methods [15,16].

A batch of 10 peacock fresh eggs were collected, were breaked to open the yolk was separated from other contents of the egg. This yolk content was pooled and homogenized with four volumes of 0.1 M sodium acetate buffer of pH 5.0. The content was centrifuged at 10,000 rpm for 20 min at 0^0C in a cold centrifuge. The supernatant was collected carefully and DEAE-Sephadex was added. After that cleared the contents in order to purify the contents, once again the DEAE-Sephadex was added and these elutents were kept for stirring for over night at 4^0C. On the next day the content were filtered with the suction filter. The protein content get bounded by the DEAE-Sephadex was washed with sodium acetate buffer for 5 times to purify the proteins. Later the protein was eluted with 0.1M sodium acetate buffer containing 0.5 M NaCl. Then the protein was dialyzed against distilled water for 12 hrs to get the semi purified protein. This semi purified protein along with DEAE-Sephadex was packed onto the column (2 x 26 cms) ion exchange chromatography. The eluted protein fraction have been collected and measured the absorbance at 280 and 455 nm (Fig. 1) were pooled and dialyzed.

The partially purified yolk riboflavin binding protein was dissolved in 1 ml of phosphate buffer and loaded onto Sephadex G-100 column (2 x 42 cm) of chromatography. The protein was eluted with 0.05 M phosphate buffer (pH 7.4) containing 0.5 M NaCl (Fig. 2). The protein fractions were collected and estimated as described by Lowry [17] (Fig. 3). The absorbance of the protein was done at 280 and 455nm. The presence of the protein and its purity was confirmed using analytical Polyacrylamide gel electrophoresis as described earlier [18].

RESULTS

The elution profile fraction was yellow in colour with the highest absorbance at 280 nm.

Spectral studies:

The absorption spectra of the partially purified peacock egg yolk RfBP and purified RfBP were shown in Figures 4 and 5. The holoprotein with the bound riboflavin showed absorption maximum at 455 nm and similar to absorption spectra were reported earlier for the flavo protein complexes [5, 19].

SDS-PAGE Electrophoresis

SDS-PAGE was carried out according to the method of Leammali [18]. (1970). SDS-gel electrophoresis of the riboflavin binding protein fraction obtained from gel filtration on Sephadex G-100 resulted in a single band on the cylindrical gels suggesting complete purification of Riboflavin binding protein. Comparison of the mobilities of molecular weight marker with the mobility of peacock egg yolk riboflavin binding protein had a molecular weight of approximately 29,000 Kda (Fig. 6).

DISCUSSION

In the present study riboflavin binding protein (RfBP) was purified for the first time peacock egg-yolk. This avian model could be used for the detailed study of the regulation of RfBP production and secretion by liver under different physiological conditions. These studies were largely restricted to the major yolk proteins like vitelloglobin [20].

Initially, RfBP from egg-yolk was purified [6, 15, and 16]. In the present study these methods were slightly modified to purify RfBP from peacock egg-yolk. Presence of large amount of lipids and other proteins in RfBP of egg-yolk makes purification more difficult. In fact [6] extracted the egg-yolk suspension with ether to remove lipids before employing the ammonium sulphate precipitation step. Further, during the purification of egg-yolk biotin binding protein, White [21] prepared an egg-yolk acetone dried powder to remove the most of the lipids. In the present study purification of peacock egg-yolk RfBP was accomplished using two steps (1) Partial purification of RfBP using DEAE-Sephadex (2) Gel-filtration on Sephadex G-100.

Initially purification of RfBP could be accomplished by batch adsorption of peacock egg-yolk homogenate to DEAE-Sephadex followed by a column elution. Gel electrophoresis of this fraction

revealed the presence of one major protein band which had mobility comparable to that of the RfBP and minor protein band. Further purification using sephadex G-100 column chromatography resulted in the complex purification of yolk RfBP homogeneity as revealed by SDS Polyacrylamide gel electrophoresis. The molecular weight appeared to be near the same that of the standard molecular weight marker (Mwm).

The partially and completely purified peacock egg-yolk was also characterized by recording the absorption spectra (Figure 5,6). The near ultraviolet absorption spectrum of the riboflavin apoprotein complex indicated that the protein had an absorption maximum at 274.3 nm and a shoulder at about 294 nm. This result is in agreement with the earlier data [22]. Further the visible absorption spectra revealed that the RfBP had absorption maxima at 370 nm and 450 nm characteristic of riboflavin apoprotein form (holoprotein). The free riboflavin showed absorption maxima at 374 nm and 455 nm (Fig. 7). Binding of riboflavin to the protein (holoprotein) resulted in the shift of the absorption peak at 445 and 457 nm and shoulders appeared at about 435 nm and 480 nm. Exactly similar spectral data was reported earlier [5, 19].

CONCLUSION

Earlier it was felt that the purification of yolk riboflavin binding protein (RfBP) was difficult due to the presence of large amount of lipids and other proteins. In fact, the egg-yolk suspension may be extracted with ether or acetone and air dried powder was prepared to remove the most of the lipids. In the present study peacock egg-yolk was isolated using (a) Batch adsorption on DEAE-Sephadex and (b) gel filtration column chromatography. Final purification by molecular sieve chromatography yielded homogenous preparation that migrated as a single band on SDS-PAGE cylindrical gel. This method was suitable for processing of protein purification and characterization studies.

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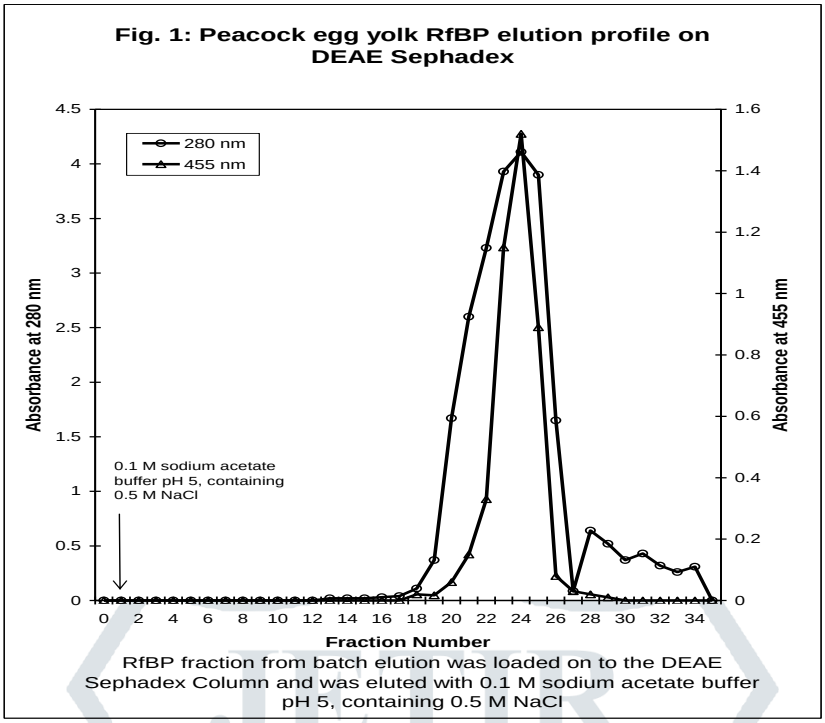


Fig. 1: Peacock egg yolk RfBP elution profile on DEAE Sephadex
280 nm
455 nm
0.1 M sodium acetate buffer pH 5, containing 0.5 M NaCl
Absorbance at 280 nm
Absorbance at 455 nm
Fraction Number
RfBP fraction from batch elution was loaded on to the DEAE Sephadex Column and was eluted with 0.1 M sodium acetate buffer pH 5, containing 0.5 M NaCl

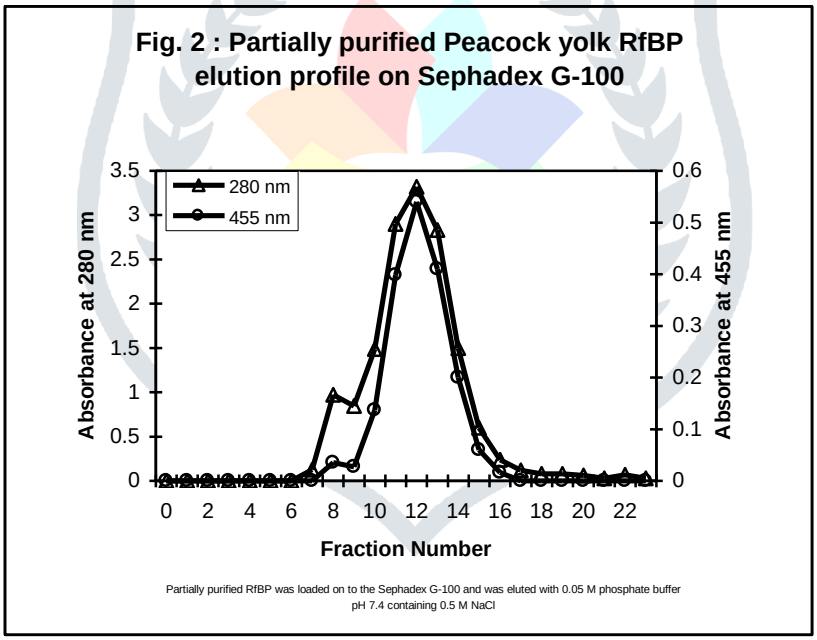
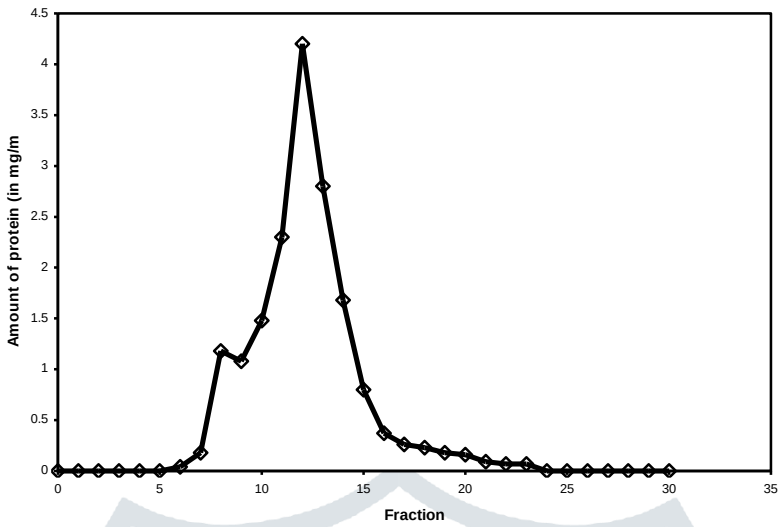


Fig. 2 : Partially purified Peacock yolk RfBP elution profile on Sephadex G-100
280 nm
455 nm
Absorbance at 280 nm
Absorbance at 455 nm
Fraction Number
Partially purified RfBP was loaded on to the Sephadex G-100 and was eluted with 0.05 M phosphate buffer pH 7.4 containing 0.5 M NaCl

Fig. 3: Peacock egg yolk RfBP elution profile on Sephadex G-100



The partially purified RfBP was eluted from Sephadex G-100 using 0.05 M phosphate buffer pH 7.4, containing 0.5 M NaCl. The protein concentration was estimated by the method of Lowry *et al* .(1951).

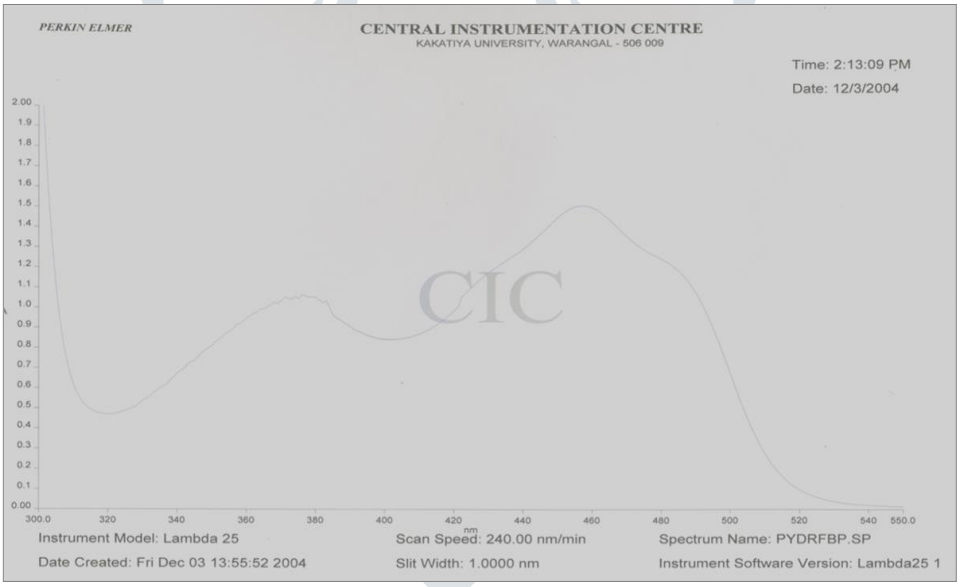


Fig. 4: Absorption spectrum of partially purified Peacock egg yolk Riboflavin binding protein (DEAE-Sephadex fraction)

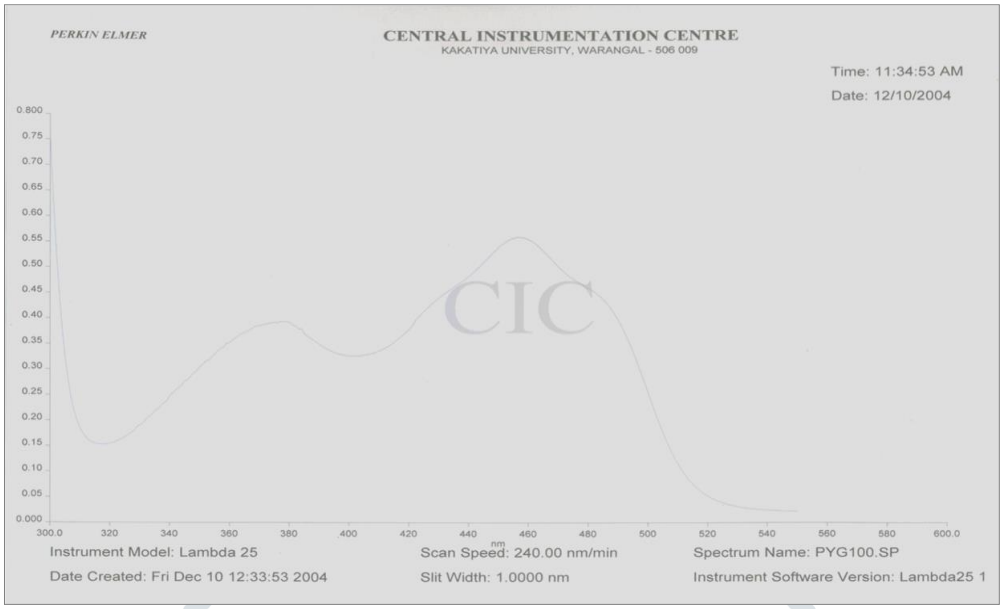


Fig – 5: Absorption spectrum of purified Peacock egg yolk riboflavin binding protein (Sephadex G-100 fraction)

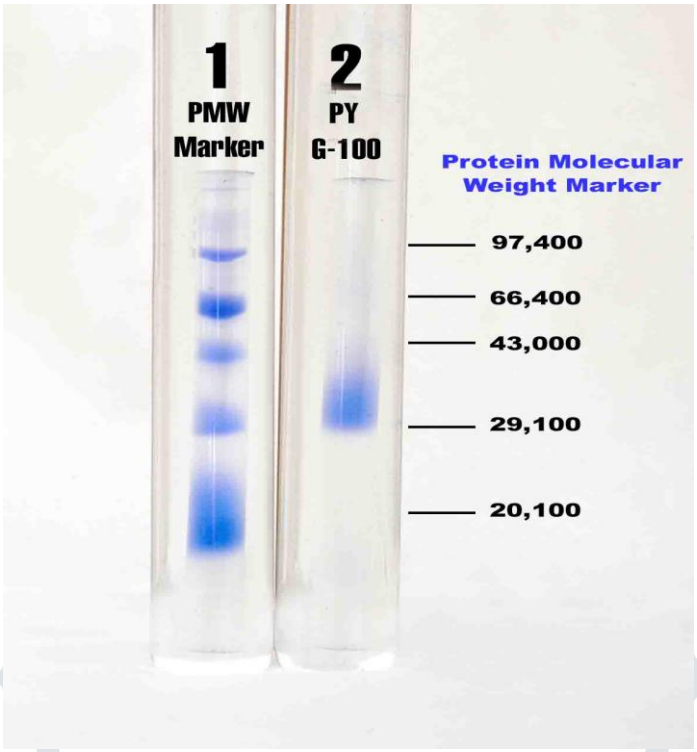


Fig. 6: Cylindrical Gel Electrophoretic pattern of the Peacock RfBP

1. Protein Molecular Weight Markers (20,000 to 97,400 kilodaltons)
2. Peacock egg-yolk RfBP Sephadex G-100 fraction

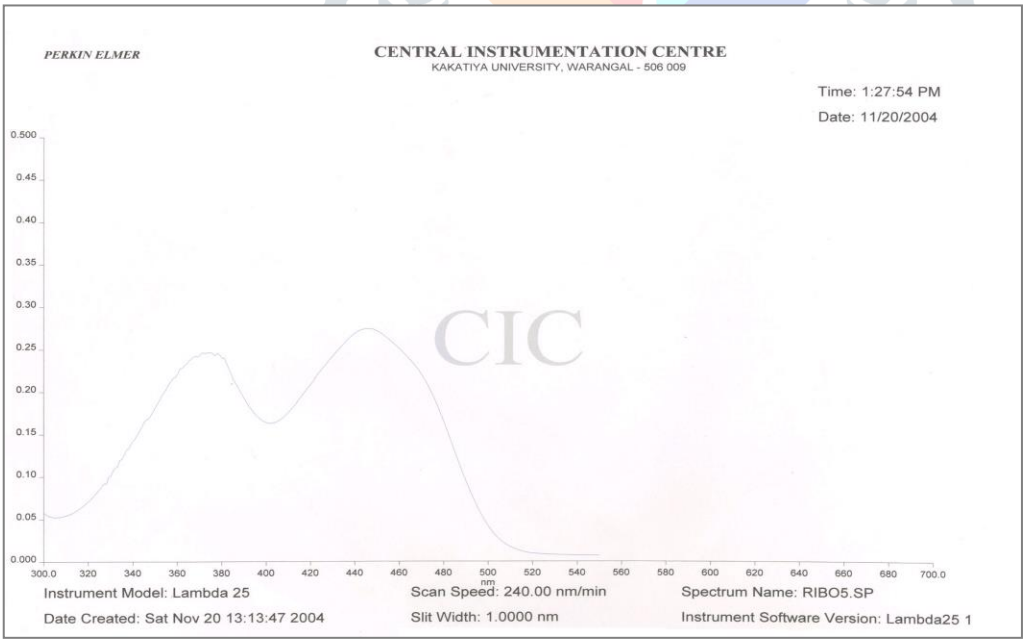


Fig - 7: Absorption spectrum of free Riboflavin (in aqueous media)