Purification and characterization of riboflavin binding protein in egg white of peacock (*Pavo cristatus*)

Animals are incapable of synthesizing the isoalloxazine skeleton of riboflavin (Rf). This vitamin is required in the range of 1–10 μg/g in the diet[1]. All flavins are 10-substituted derivatives of the isoalloxazine tricycle ring system which is synthesized via a complex pathway from GTP[2]. In mammals, Rf [7,8-dimethyl-10-(1-D-ribityl isoalloxazine)] is found in urine and milk. However, it is also found in eggs of reptiles and birds. The two coenzymatic derivatives of Rf, flavin mononucleotide (FMN; Rf 5′-phosphate) and flavin adenine dinucleotide (FAD; Rf 5′-adenine diphosphate) function as prosthetic groups in several mitochondrial oxidative–reduction enzymes. The specific binding proteins for fat-soluble vitamins such as vitamins A and D have been identified in the serum of all vertebrates[3–7].

The binding proteins for water-soluble vitamins[7,8], vitamin B_{12} (refs 9 and 10) and thiamin[11,12] have been found in the blood serum, egg white and egg yolk of the egg-laying hens. The essential role of Rf-binding protein (RfBP) has been demonstrated in the homozygous recessive mutant (rd-rd) domestic fowl[13] and in heterozygous leg horn hen[14].

In the present study an attempt has been made to isolate RfBP in peacock egg white. Peacock eggs were collected from Gopalpur hillock located 10 km away from Kakatiya University campus, Warangal District, Andhra Pradesh, which is a tropical region. The DEAE-Sephadex A-50 was obtained from Pharmacia Fine Chemicals (Sweden). Sephadex G-100 and Freund's complete adjuant were procured from Sigma–Aldrich (USA). Bovine serum albumin acrylamide *N,N,N′,N′*-tetramethylethylene diamine, *N,N′*-methylene-bis-acrylamide and sodium dodecyal sulphate were obtained from Loba Chemicals (Mumbai).

The RfBP was isolated following the methods described earlier[7,14,15]. Peacock egg white RfBP was purified to apparent homogeneity in two steps: batch adsorption to DEAE-Sephadex and gel filtration column chromatography on Sephadex G-100 (refs 15, 16). The protein content in all fractions was estimated[17]. The peak fractions were then pooled and dialysed.

To the solution of peacock egg white, 0.1 M sodium acetate buffer, pH 5.0 was added and run on a DEAE-Sephadex column. The fraction containing 0.5 M NaCl eluted the RfBP concentration showed absorbance at $A_{280\ nm}$ and also at 455 nm (Figure 1). The polyacrylamide gel at pH 7.4 of this protein fraction suggested that the protein is partially purified at this stage (Figure 2). The protein peak fraction from DEAE-Sephadex was lyophilized after dialysis. A solution of this fraction was loaded onto Sephadex

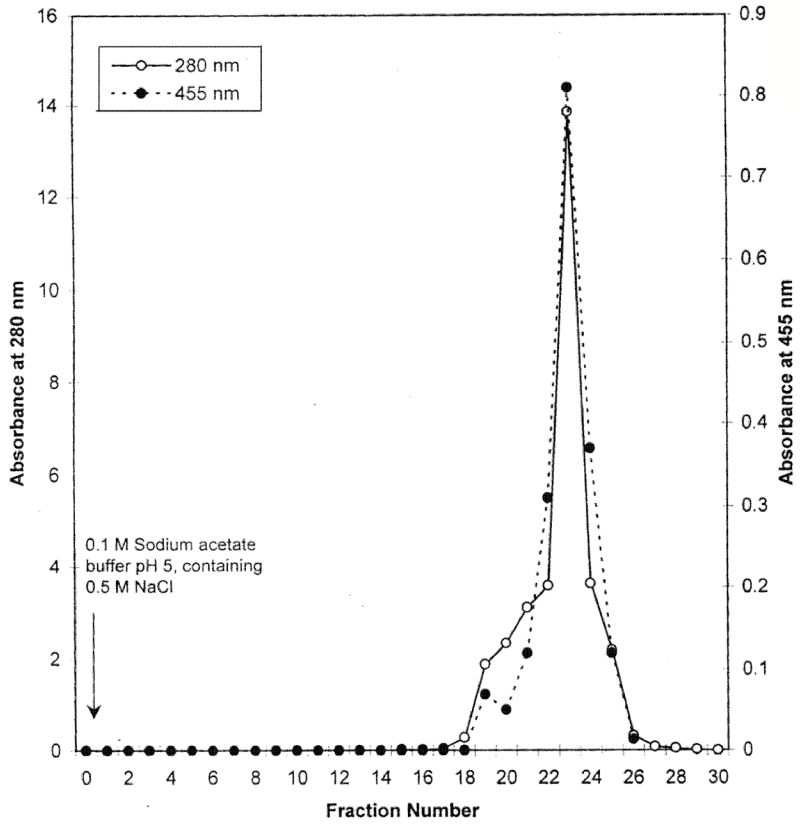

Figure 1. Peacock egg white RfBP elution profile on DEAE-Sephadex.

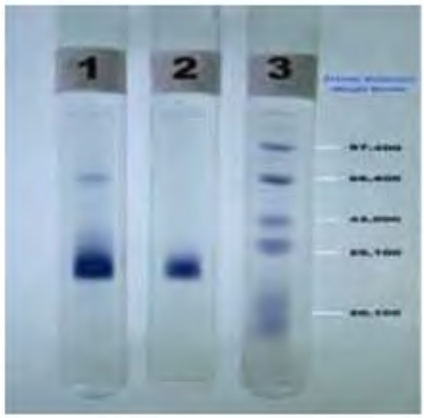

Figure 2. Cylindrical gel electrophoretic pattern (SDS–PAGE). Lane 1, Peacock egg white DEAE-Sephadex elution fraction. Lane 2, Peacock egg white Sephadex G-100 fraction. Lane 3, Protein molecular weight marker (20,000–97,400 kDa).

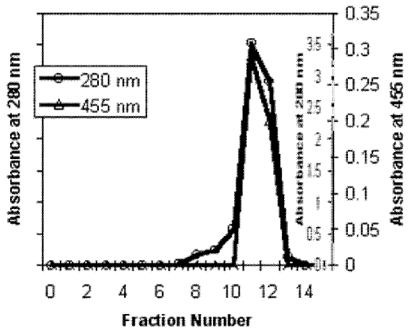

Figure 3. Partially purified peacock egg white RfBP elution profile on Sephadex G-100.

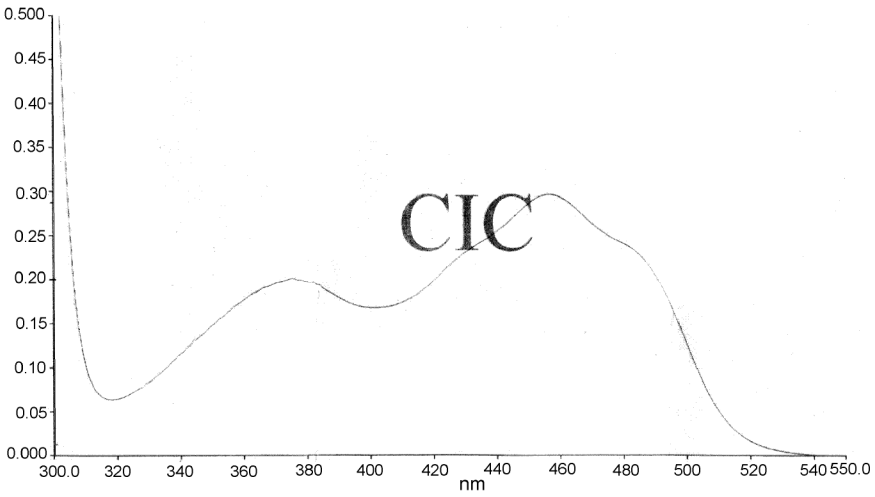

Figure 4. Absorption spectrum of peacock egg white RfBP (Sephadex G-100 RfBp fraction).

G-100 column and eluted with 0.05 M phosphate buffer, pH 7.4, containing 0.5 M NaCl; twenty fractions were collected. Fractions 11 and 12 have absorbance at 280 and 455 nm (Figure 3). The UV absorbance spectrum of this fraction showed typical two-banded spectrum of Rf, with maximum absorbance at 375 and 455 nm. This is in agreement with absorption spectra of flavoprotein complexes[7,18]. The purity as judged by native PAGE (Figure 4) and SDS–PAGE[19] using slab gel and cylindrical gel respectively, gives the homogeneity of RfBP, as it moved as a single band on the both gels.

It may be mentioned that this is an initial attempt on the isolation and purification of RfBP of peacock egg white, and better purification could be achieved using successive column-chromatography binding steps, i.e. DEAE-Sephadex and Sephadex G-100. Further, the purified peacock egg white RfBP migrated as a single band during electrophoresis on SDS–PAGE. The molecular weight of this protein is around 29,000 kDa.

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ACKNOWLEDGEMENTS. We thank the technical staff of the Central Instrumentation Centre, Kakatiya University, Warangal for assistance in spectral studies. We also thank the Head, Department of Biochemistry and Zoology, Kakatiya University for providing necessary laboratory facilities.

Received 27 June 2006; revised accepted 29 March 2007

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