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PURIFICATION AND CHARACTERIZATION OF RIBOFLAVIN BINDING PROTEIN (RfBp) FROM HEN (*Gallus gallus*) EGGS USING DEAE-SEPHAROSE COLUMN CHROMATOGRAPHY

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ABSTRACT : Riboflavin binding protein (RfBP) was isolated, purified and characterized from Hen (*Gallus gallus*) egg white and yolk using Sepharose column chromatography. The Rfbp was purified using DEAE-Sepharose ion exchange chromatography followed by gel filtration on Sephadex G-100. The protein content was estimated with Lowry method. The purity of the proteins was judged by SDS-PAGE technique. The protein migrated as a single band on SDS gel with a molecular weight of 29 kilodaltons. This is the first report on purification of this protein using DEAE-Sepharose column chromatography.

Keywords: Hen eggs, RfBp, Purification.

INTRODUCTION

Riboflavin Binding Protein (RfBP) or Riboflavin Carrier Protein (RCP) was first isolated from the chicken egg white (Rhodes *et al*., 1959). Egg white RfBP is a phosphoglycoprotein having a molecular weight of 29,200 containing 219 amino acid residues (Hamazume *et al*.,1984). The isolation of RfBP from egg yolk was first published (Ostrowski *et al*.,1962) and improved methods were subsequently reported (Miller ,1981). The Riboflavin binding protein from peacock (*Pavocristatus*) egg-white was first isolated and purified by Rajendar *et al*. (2007, 2009). The essential role of Rfbp has been demonstrated from a study of the homozygous recessive mutant (rd rd) of domestic fowl (Winter *et al*., 1967). Developing embryos having this genetic constitution die at around 13 days of incubation, from riboflavin deficiency. Subsequently, it was shown that the homozygous recessive (rd rd) hens were unable to synthesize riboflavin binding protein (Winter *et al*., 1967). The aim of the present study was to purify the Riboflavin binding protein from hen egg white and yolk using DEAE-sepharose column chromatography which was not reported earlier

MATERIALS AND METHODS

Fresh hen (*Gallus gallus*) eggs were obtained from the poultry farm, Waddepally, Warangal. DEAE-Sepharose, and Sephadex G-100 were obtained from Sigma-Aldrich Chemical Company (St. Louis, USA).

Bovine Serum albumin, acryl amide, N, N, N1, N1-Tetramethylethylene-diamine, N, N1-methylene-bisacrylamide, SDS were procured from Loba Chemical Industrial Company, Bombay, India. All other reagents used were of analytical grade. Riboflavin binding protein from hen egg-white and yolk 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. Protein content was estimated by the method of Lowry (1951).

RESULTS AND DISCUSSION

Proteins from the eggs of different avian species have been studied by many researchers, the most extensive of all was the ovomucoid (Laskowski *et al*., 1987). The isolation of RfBP, purification and characterization of the flavoprotein apoprotein system of chicken egg white was first reported (Rhodes *et al*., 1958). Since then, several variations in the isolation procedures were based on the tight binding of the protein to DEAE-cellulose, CM-cellulose or SE-Sephadex A-50, DEAE-Sephadex followed by gelfiltration chromatography on Sephadex G-100. RfBp from hen egg white and yolk was completely purified to homogeneity using DEAE-Sepharose at pH 5.5, followed by gel filtration using Sephadex G-100. The fractions were monitored for absorbance at 280 nm and 455 nm(fig.1). The peak fractions (18 to 24th) which were yellow in color with highest absorbance at 280 nm were pooled, dialyzed against distilled water and lyophilized. The protein showed a molecular weight of 29 kilodaltons (fig.2) on SDS-PAGE.

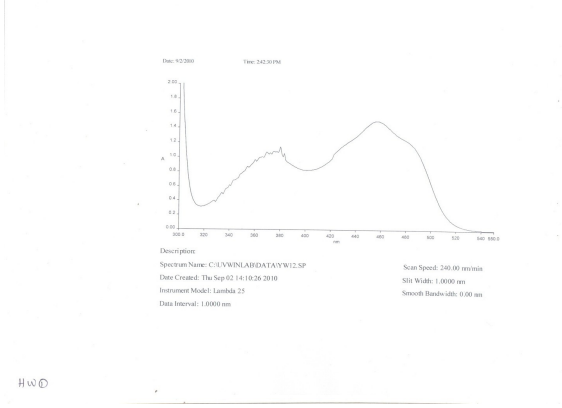

Figure 1 : Fraction absorbance

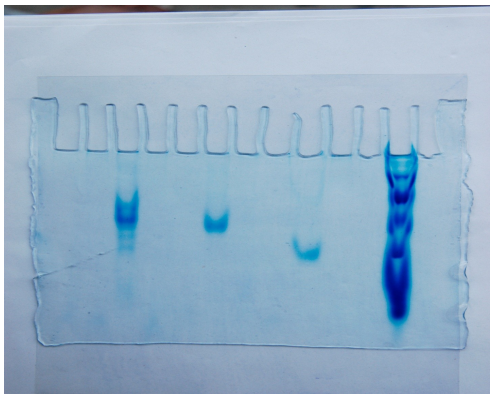

Figure 2 : SDS-PAGE

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