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Isolation, Purification and Characterization of Riboflavin Carrier Protein (RCP) from Emu Egg Yolk (*Dromaius novaehollandia*)

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Abstract

Riboflavin carrier (or binding) protein (RCP) was isolated from the Emu egg yolk (*Dromaius novaehollandia*) for the first time. In the present study an attempt has been made to isolate and purify the RCP in two steps, DEAE Sephadex A-50 ion exchange chromatography and the final purification was achieved on sephadex G-100.The purity of the protein was judged on cylindrical and slab gel electrophoresis,SDS-PAGE technique. Sephadex G-100 re-chromatographic fraction RCP moved as a single band both on the slab and cylindrical gels. Comparison of the mobility of the RCP isolated from Emu egg yolk with that of the standards molecular weight marker proteins revealed that the molecular weight of the protein is higher than 29 kDa.

Keywords: Emu egg, Purification, RCP, SDS-PAGE.

Introduction

Riboflavin (Rf) is a water soluble vitamin essential for normal cellular function, growth and development. This vitamin is required in the normal range of 1-10µg/g in diet (Dadd, 1958).The two co-enzymatic derivatives of Rf, flavin mono nucleotide (FMN; Rf 5'-phospate) and flavine adenine dinucleotide (FAD; Rf 5'-adeninediphosphate) acts as a prosthetic group in several mitochondrial oxidative-reduction enzymes. It was reported that most of the vertebrate tissues contains predominantly FAD (ca. 75% of the total tissue flavine), followed by FMN (ca. 22%) and Rf (ca. 2%) (Yagi and Maruyama, 1971). It has been shown in recent studies that

transportation of these vitamins to the growing embryo is due to specific carrier proteins. The carrier proteins for the water soluble vitamins (Rhodes et al ., 1959; Ostrowski et al.,1962:), vitamin B12 (Grasbeck, 1969: Sonneborn and Hansen, 1970) and thiamine (Naber et al 1954: Coates ,1971) has been found in the blood serum , egg white and egg yolk of egg laying avianes. The specific binding proteins for fat soluble vitamins such as vitamine A and vitamine D are identified in normal serum in all vertebrates (Kanai and Goodman ,1968: Abe et al ., 1975: Thomas et al ,1959: Edelstein et al ,1973) .Riboflavine carrerier priotein (RCP) are riboflavibne binding protein (RfBP) was first isolated from hens egg white by Rhodes et al 1959. The egg white RfBP is monomeric polypeptide containing 219 aminoacid residues whith a molecular weight of 29,200.(Hamazume et al 1984). The RfBp from egg yolk was first isolated Ostrowski etal 1962 and many improved method were subsequently reported by Miller 1981 and Murthy et al , 1979. The essential role of RfBP or RCP has been demonstrated in the homozygoeous recessive mutants (rd-rd) domestic fowl (Winter et al ., 1967) and in the hereterogygeous leg hornhen by Farell etal ., 1907. In the present study an attempt has been made to isolate RfBP in *Dromaius novaehollandiae*.(Emu) egg yolk.

Material and methods

Fresh Emu (*Dromaius novaehollandia*) eggs were obtained from the poultry fram, madikonda, Warangal. DEAE-Sephadex A-50 was obtained from pharmacia fine chemicals (Sweden0. Sephadex G-100 was obtained from sigma-Aldrich chemical company (St.louls, USA). Bovine serum albumin, acrylamide N,N ,N,N Tetramethyleneethylenediamine, N,N -methylene-bis-acrylamide and sodium dodecyl sulphate were obtained from Loba chemical industrial company,Mumbai,India. All other reagents used were of analytical grade.

RCP was isolated from Emu egg yolk following the methods of Rhodes *et al*., 1959: Farrel *et al*., 1970; Hamazume *et al*., 1984 and Murthy *et al*., 1979 with little significant modifications. The protein estimations were done by using the method of Lowry *et al*., 1951. SDS-PAGE was carried out according to the method Leammli, 1979 using sodium phosphate buffer containing SDS.

Results

Emu egg yolk RCP was purified to apparent homogeneity in two steps. Batch absorption to DEAE-Sephadex A-50 and gel filtration column chromatography on sephadex G-100. First Emu egg yolk was homogenized with four volumes of sodium acetate buffer pH 4.6. The crude yolk suspension was centrifuged at 15,000 Xg for 30 min at 0°C. The clear supernatant was left for 12 hr in cold condition. The clear yellow supernatant was directly used for batch absorption on the DEAE-Sephadex A-50. The DEAE-Sephadex A-50 with bound proteins was washed extensively with the same buffer. Then eluted with 0.1 M sodium acetate pH4.6 containing 0.5 M NaCl. The eluted samples were loaded on the column (2 X 36) of DEAE-Sephadex A-50 and washed with excess of the same buffer and RCP was eluted with 0.1M sodium

acetate buffer containing 0.5M NaCl. The fractions were collected and the absorbance were measured at 280 nm and 455 nm using UV-Spectrophotometer (Fig 1).The polyacrylamide gel at pH 7.4 of this peak protein fraction suggested that the protein is partially purified at this stage (Fig 2). The protein peak fraction from DEAE-Sephadex A-50 was lyophilized after dialysis.

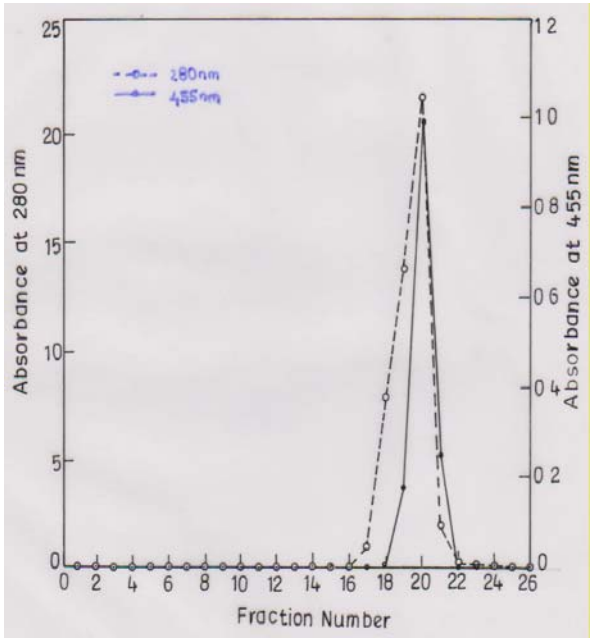


Figure1: Emu egg yolk RCP elution profile on DEAE-Sephadex.

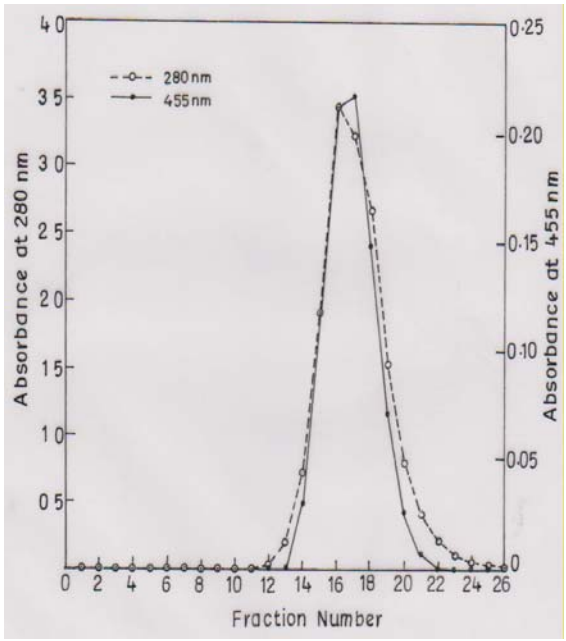


Figure2: Partially purified Emu egg yolk RCP elution profile on Sephadex G-100.

Further purification was achieved by gel filtration column chromatography on Sephadex G-100 and eluted with 0.05 M phosphate buffer pH 7.4 containing 0.25M NaCl. Twenty six fractions were collected, and absorbance were collected at280 nm and 455nm (Fig 3). As the eluted protein from the Sephadex G-100 was not totally free from the contamination proteins an additional re-chromatographic step on DEAE-Sephadex was under taken. The contamination proteins were removed by washing the DEAE-Sephadex column with 0.1 M NaCl and 0.15 M NaCl before eluting the RCP with 0.5M NaCl containing buffer. The UV absorbance of the peak fraction showed absorbance peaks at 379 nm and 458 nm (Fig 4). From the PAGE pattern (Fig 5) it could be seen clearly that Emu egg yolk was purified to and apparent homogeneity and the molecular weight is slightly higher than 29,000. (Fig 6)

Figure3: Cylindrical gel electrophoretic pattern of the native partially purified Emu egg yolk RCP.

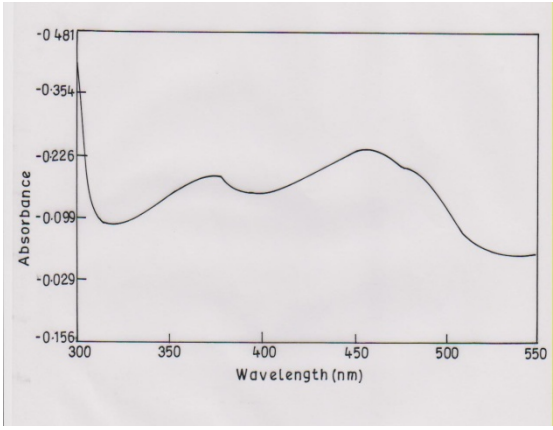


Figure4: Absorption spectrum of Emu egg yolk RCP (Sephadex G-100 RCP fraction).

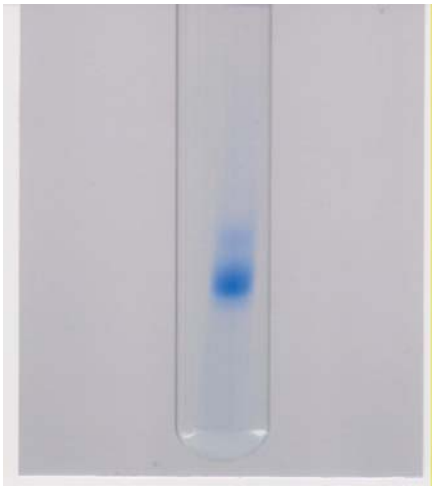
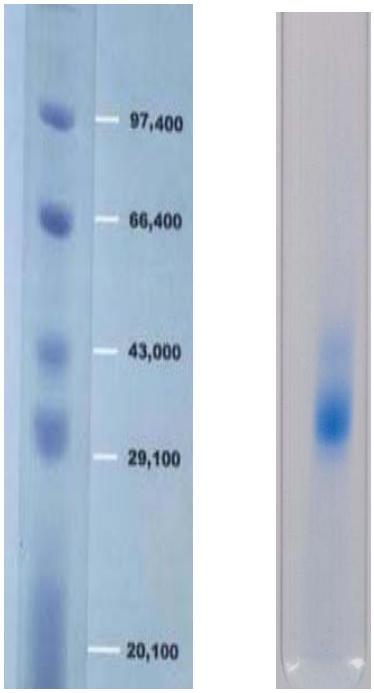


Figure5: SDS-PAGE pattern of purified Emu egg yolk RCP (Rechromatographic fraction).



A B

Figure6: SDS-PAGE pattern of purified Emu egg yolk RCP (A) and protein molecular weight markers (97 kDa-20 kDa) (B).

Discussion

In the present study, Riboflavin carrier Proteins (RCP) was purified, for the first time, from Dromaius Novaehollandiae (EMU) egg yolk. The long range goal of this study

is to develop an avian model for the detailed study of the regulation of RCP production and secretion by liver under different pathophysiological conditions. Earlier, considerable attention was focused on the estrogen-induced synthesis of specific egg yolk proteins in avian and amphibian livers as model systems. However, these studies were largely restricted to the major yolk proteins like vitellogenin (Gruber M., *et.al*., 1976). Relatively, very little is known regarding the other important, albeit minor, yolk proteins such as the vitamin binding proteins. So, an attempt has been made to isolate RCP from a flight less bird Dromaius Novaehollandiae (EMU) egg yolk and characterized the molecular weight as little higher than 29 kDa.

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