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Isolation and amino acid composition of Pigeon (*Columba livia*) egg white and egg yolk Riboflavin carrier protein

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Riboflavin carrier protein (RCP) from pigeon egg white and egg yolk have been isolated. The molecular weight of purified RCP of pigeon egg white and yolk has been found to be nearly same as that of previously isolated hen egg white and yolk RCPs. The amino acid composition of pigeon RCPs were observed to be almost similar to that of hen RCPs.

Key words : Riboflavin Carrier Protein, Amino acid composition, Purification,*Columba livia.*

INTRODUCTION

Riboflavin carrier protein (RCP) is present in both avian egg yolk and white, in contrast reptilian eggs it is only present in the yolk (Rhodes et al 1959 and White and Merrill 1988). RCP has an important role in the uptake of riboflavin to the developing oocyte. In mutants of the domestic fowl lacking RCP, the developing embryos die of riboflavin deficiency at around 13 days of incubation (Winter et al 1967). At this stage a rapid increase occurs in flavin kinase activity, requires for the synthesis of FMN and FAD17. RCPs from hen egg white, yolk and plasma have been purified and sequenced (Norioka *et al.*, 1985). All the three proteins are the products of the same gene, although plasma and egg yolk RCPs are synthesized in the liver and egg white RCP is in the oviduct. All the three show polymorphism at 14th position. Egg yolk RCP differs from the other two having 11-13 fewer amino acid residues. When the plasma RCP is taken up into the egg yolk the C-terminal peptide is cleaved. There is also some difference in the glycosylation pattern, although in all the three the oligosaccharides are linked by asparagines 36 and 147 (Miller *et al.*, 1982).

Little detailed characterization has been carried out on any RCPs from other avian sources. The purified RCP from duck egg white has a quite distinct amino acid composition from that of domestic fowl (Muniyappa and Adiga1980). Because of the difference in amino acid composition of egg white RCPs reported for duck and domestic fowl, a wide range of species needs to be investigated in order to establish the common features and differences.

In the present study RCPs from egg white and yolks of pigeon and hen are isolated and pigeon egg white yolk amino acid compositions are analyzed.

MATERIALS AND METHODS

Pigeon eggs were obtained from the local source. DEAE-Sephadex A–50 used in the present study was obtained from Pharmacia Fine Chemicals, Uppasala, Sweden; Sephadex G - 100 from Sigma Chemical Company, St. Louis, USA. Bovine serum albumin, acrylamide N, N, N^1, N^1-Tetramethylethylenediamine, N, N^1-methylene-bis-acrylamide, SDS were procured from Loba Chemical Industrial Company, Bombay, India. All other reagents used were of analytical grade.

Pigeon egg whites and yolks were carefully separated and used immediately. RCP from pigeon egg white was isolated following the methods (Farrell *et al.*, 1969, Hamazume 1984 and Rhodes *et al.*, 1959) with a few modifications as described below. Pigeon egg white was homogenized with an equal volume of 0.1M sodium acetate buffer, pH 4.5; the precipitated protein was removed by centrifugation. To the clear supernatant, DEAE sephadex previously equilibrated with 0.1M sodium acetate buffer pH 4.5 was added. The mixture was stirred for 12 hours at 4 ^{0}C and then suction filtered. The DEAE sephadex with bound protein was washed with excess of 0.1M sodium acetate buffer pH 4.5. Bound proteins were eluted with same buffer containing 0.5M sodium chloride by suction filtration. The eluted protein fraction was dialyzed against water.

Fresh DEAE sephadex, previously equilibrated with

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0.1M sodium acetate buffer pH 4.5 was packed into the column and then the partially purified RCP was loaded onto the column. The RCP was eluted from the column with the same buffer containing 0.5M sodium chloride, Fractions were collected and absorbance was measured at 280nm and 455nm using uv visible recording spectrophotometer. Values were expressed as total absorbance at 280nm and 455nm per each fraction. The peak fractions are pooled and dialyzed against distilled water and lyophilized.

Further purification of pigeon egg white RCP was achieved by gel filtration column chromatography using sephadex G-100. The protein was loaded on the column and eluted with the 0.025M phosphate buffer pH 7.3 containing 0.5M sodium chloride. Protein in each fraction was determined by the method (Lowry et al 1951). Absorbance was measured at 280nm and 455nm using uv visible recording spectrophotometer. Values were expressed as total absorbance at 280nm and 455nm per each fraction. The peak fractions are pooled and dialyzed against distilled water and lyophilized. The purity of the protein was checked by SDS-PAGE. Pigeon egg yolk RCP was also purified with significant modifications to apparent homogeneity in two steps: batch absorption to DEAE sephadex and Gel filtration column chromatography on sephadex G-100. SDS-PAGE was carried out according to the method of Leammli using Tris-Glycine buffer containing SDS. 7.5 % gels were used. The absorption spectrum was recorded using UV-visible spectrophotometer (UV 160A, SHIMADZU).

Amino acid composition of the riboflavin binding protein was analyzed in Beckman HPLC amino acid analyzer, model no. 119 CL. Amino acid composition of riboflavin Binding Protein was determined by hydrolysis of the protein. Approximately 4.99 mg of pigeon egg white and 1.36mg of pigeon egg-yolk RCP was taken and dissolved in 5ml of 6N HCI and hydrolyzed in a hot air oven at 110 ^{0}C for 22 hours. After the hydrolysis, the contents were made up to 10ml. 5ml of the aliquot were taken and flash evaporated to dryness. The HCI was removed like this by evaporating 3 to 4 times. Then 2 ml of Lithium Citrate buffer pH 2.2 was added to the residue. 100 ml of the sample was loaded on to the column. The amino acid contents were calculated with respect to the standard amino acid mixture.

RESULTS AND DISCUSSION

It was found that better purification could be achieved using two successive ion exchange binding steps. Nearly homogenous preparation of RCP was obtained at this stage of purification, which was revealed by SDS-PAGE as shown in Fig. 1.

i) Purified pigeon egg white RCP ii) Purified pigeon egg yolk RCP :

However, final purification was accomplished using gel

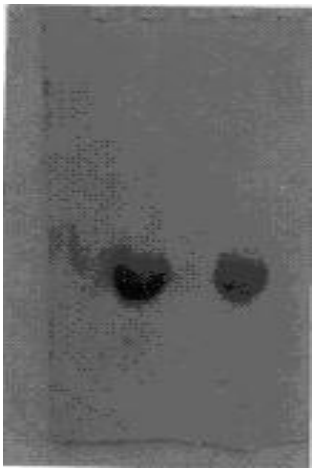
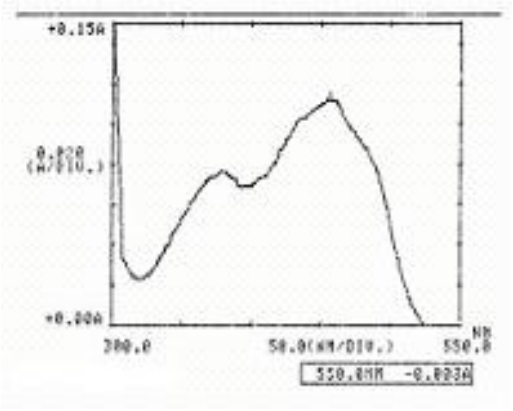


Fig.1: SDS-PAGE pattern of purified pigeon egg white and yolk RCPs



a) Pigeon Egg White RCP

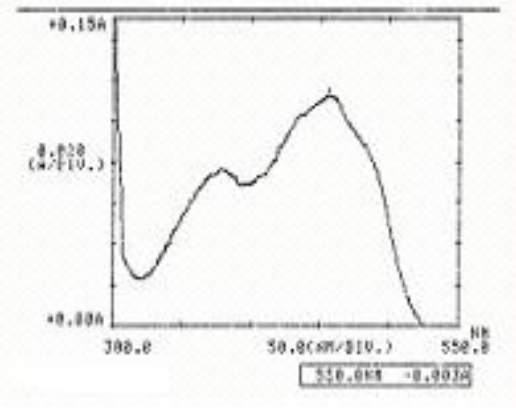


Fig. 2: The absorption spectrum of purified pigeon RCP

filtration on sephadex G-100. RCPs constituted less than 1% of total proteins in egg white, and adopting this two stage purification method RCP could be purified to homogeneity.

It was found that better purification could be achieved using two successive ion exchange binding steps. Nearly homogenous preparation of RCP was obtained at this stage of purification, which was revealed by SDS-PAGE as shown in Fig.2. The molecular weight of pegion egg white and yolk appeared to be nearly the same as that of the hen egg-white and yolk RCP.

The near uv absorption spectrum of the riboflavin-apoprotein complex indicated that the protein had a absorption maximum at 274.3nm and a shoulder at about 290nm. This result is in full agreement with the data published (Rhodes et al 1959). Further the visible absorption spectra revealed that the RCPs had absorption maxima at 370nm and 456nm, characteristic of riboflavin-apoprotein forms (holoproteins). The free riboflavin showed absorption maxima at 374 and 445nm. Binding of riboflavin to the protein resulted in the shift of the absorption peak at 445nm to 457nm and shoulders appeared at about 435 and 480nm. The uv absorption spectra of purified pigeon egg white and yolk RCPs is shown in Fig. 2a and 2b.

Table 1: Amino Acid content of purified pigeon egg white and yolk RCP

Amino acid	µg AA/100µg protein	
	Pigeon egg white	Pigeon egg yolk
Aspartic acid	8.48	8.90
Threonine	4.91	4.94
Serine	4.87	7.43
Glutamic acid	7.98	9.16
Proline	2.68	4.00
Glycine	2.72	2.50
Alanine	3.72	4.22
½ Cystine	Nd	Nd
Valine	4.13	4.26
Methionine	Nd	Nd
Isoleucine	3.20	3.90
Leucine	6.97	6.04
Tyrosine	4.12	3.42
Phenylalanine	5.13	5.04
Lysine	3.67	3.98
Histidine	1.96	3.02
Arginine	6.48	6.86

The amino acid compositions of pigeon egg white RCP and pigeon egg yolk RCP have been shown in Table – 1. The amino acid composition appears to be nearly similar but yolk RCP apparently contain more serine, and slightly higher glutamic acid than that of egg white RCP. Furthermore comparison of amino acid composition on the whole between hen and pigeon revealed striking similarities.

The amino acid composition of pigeon egg white and yolk RCP is shown in Table-I. It was observed that the amino acid composition of pigeon egg white RCP has close similarity with hen egg white RCP.

The amino acid composition of duck egg white RCP was initially reported by Muniyappa and Adiga 1980. They found significant differences in the amino acid composition when compared with hen egg white RCP. On the other hand, (Stevens et al 1994) 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 RCPs, particularly in proline, arginine, methionine, phenylalanine and histidine contents. The amino acid analysis of quail egg-white RCP and egg-yolk RCP revealed close similarities in the amino acid composition not only between quail egg-white RCP and quail egg-yolk RCP but also between quail RCPs and hen RCPs. In the present study, it was observed that the amino acid composition of pigeon egg white and yolk has close similarity with hen RCPs. This study suggests the existence of some significant structural differences between the RCPs from Pigeon and hen.

Abbrevations:

SDS PAGE, sodium dodecyl sulphate poly accrylamide gel electophoresis; RCP, riboflavin carrier protein; DEAE, diethyl amino ethyl; Tris, 2-amino 2-hydroxy methyl propane-1,3 diol; HPLC, high performance liquid chromatography; nm, nano meter.

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