*Asian J. of Bio Sci. (2006) Vol. **1** No. 2 : 157-159*

Purification and characterization of Riboflavin carrier protein from hen egg - yolk and pigeon egg - yolk

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(Accepted : June, 2006)

Riboflavin carrier protein (RCP) has been purified from both pigeon and hen egg- yolk by ion exchange chromatography and gel filtration techniques. Purity of pigeon egg- yolk and hen egg-yolk RCPs are determined by SDS-PAGE. Their structures are compared using Circular Dichroism (cd) Spectra and Fluorescence Spectra. For Pigeon egg-yolk RCP amino acid composition was analyzed. In the present study it is observed that the two proteins have similar secondary structure but they differ in their tertiary structures. The amino acid composition of pigeon egg-yolk RCP has close similarity with hen egg-yolk RCP. This study suggests the existence of some significant structural difference between RCPs from pigeon and hen.

Key words : Riboflavin Carrier Protein; Protein purification and Characterization.

INTRODUCTION

VITAMINS are essential for growth, development and metabolism of an individual. It has been shown in recent studies[2,7,14] that transportation of these vitamins to the growing embryo is due to specific carrier proteins. The importance of riboflavin carrier protein in the transport of riboflavin into the egg is particularly clear. A strain of chickens incapable of concentrating riboflavin in the egg can only be maintained by injection of riboflavin into their riboflavin-deficient eggs[16]. The defect in these chickens is the lack of a functional riboflavin carrier[15].

In the present study RCP was purified for the first time from the pegon egg-yolk and compared with hen egg-yolk RCP, with a view to develop this avian model for studying the regulation of RCP production under pathophysiologcal conditions.

MATERIALS AND METHODS

Commercially available hen (Gallus gallus) eggs were used and pigeon (Columba livia) eggs were obtained from a local source. RCP from hen egg yolk was isolated following the methods [3,8] with a few modifications as described below. Pigeon egg-yolk was homogenized with four volumes 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 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-yolk 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[6]. 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. Hen egg-yolk RCP was also purified to apparent homogeneity in two steps: batch absorption to DEAE sephadex and Gel filtration column chromatography on sephadex G-100.

SDS-PAGE:

SDS-Page was carried out according to the method of Leammli[5] using Tris-Glycene buffer containing SDS. 7.5 % gels were used.

SPECTRAL STUDIES

a) UV Spectra:

The absorption spectrum was recorded using UV-visible spectrophotometer (UV 160A, SHIMADZU). The absorption spectrum of the purified RCP preparations were recorded by diluting the proteins with 0.05M Tris-HCl buffer, pH 7.5 or directly from the eluates of the column after diluting the solutions suitably.

b) CD Spectra:

CD Spectra were recorded at 20^{0}C in a cd machine Jovine 750 Dichrograph. Far uv (190 to 260nm) and near uv (260 to 320nm) spectra were recorded. The protein concentration for far uv cd was 0.2 mg/ml and the cell path length was 0.02 cm. The protein concentration for near uv cd was 1 mg/ml and the cell path length of 1 cm were used.

c)Fluorescence Spectra:

Fluorescence spectra were recorded at 20^{0}C in 4D 10 Hitachi Spectrofluorimeter with excitation at 280nm and 295nm. The protein concentration was 1 mg/ml.

Amino Acid Composition:

Amino acid composition of pigeon egg-yolk RCP was analyzed in Beckman HPLC amino acid analyzer. The amino acid contents were calculated with respect to the standard amino acid mixture.

RESULTS AND DISCUSSION

The SDS-Page pattern of purified RfBPs from hen and pigeon egg-yolk are shown in Fig.1. The pigeon egg-yolk RCPs could be

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