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Research Article

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Prediction of riboflavin carrier protein (RCP) secondary structure using K_2D_2 web tool

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ABSTRACT

Circular dichroism spectroscopy is used to analyze the secondary structure of Riboflavin Carrier Protein (RCP). Major secondary structure types, alpha-helices and beta-strands produce distinctive CD spectra. Secondary structure of unknown structure can be accessed from the known if unknown structure CD spectrum is given. We have observed the secondary structure of Riboflavin carrier protein contain of hen, coot and spotted owlet eggs white. Similar alpha percentage and beta stand percentage was observed in hen and coot but in spotted owlet Yolk difference in alpha percentage (%) and beta stand percentage (%) was seen. The secondary structure of RCP was estimated with an error of 0.32 using web base online K_2D_2 software. We have observed that this method improves the predictions of alpha and beta strand, particularly for the wavelength interval between 190 and 240 nm.

Key words: Circular dichroism (CD), Riboflavin Carrier Protein (RCP), K_2D_2 web tool.

INTRODUCTION

Circular dichroism (CD) is a spectroscopy uses left and right circularly polarized light for studying protein, peptides and DNA metal complexes. The evaluation of the conformation and stability of proteins in several environmental conditions can be studied using this spectrum. In this paper we discuss the application of online web server for estimating structural composition of proteins. Isolated Riboflavin carrier protein (RCP) from Hen, Coot and Spotted owlet eggs CD spectra were used in this study. RCP in adequate amounts is essential for normal fetal development. Pregnancy-specific riboflavin-binding proteins have been found in rat, mice, bovine, simian and human plasma (Muniyappa and Adiga, 1980; Natraj *et al*., 1987; Merril *et al*., 1979; Visweswariah and Adiga, 1987; Murthy and Adiga, 1982b). RCP binds with riboflavin in equal molar ratio tightly and is required for the transport of riboflavin to the developing oocytes. The RCP gene is controlled by estrogen and is expressed both in the liver and the oviduct of laying hens. The inability to produce RCP in chicken is associated with embryonic death in the eggs. Hen, Coot (Madhukar Rao *et.al*., 2013 and 2015) and spotted owlet RCP (Karunakar Rao *et al*., 2012, 2013 and 2015) have been purified and characterized. The secondary structures of riboflavin protein were compared using K_2D_2 software which was earlier investigated by Carolina and Miguel *et.al*., (2008). The secondary structures of hen, coot egg yolk, white and owlet egg white, yolk of isolated RCP were compared and the results are presented. The present approach is less complicated than the currently available methods, and also sample concentration is not required. It can be very useful for the study of lower concentrations of any protein to be analyzed.

EXPERIMENTAL SECTION

The isolation and purification of Riboflavin Carrier Protein (RCP) from Hen, Coot and Spotted Owlet Eggs White and Yolk was done in the Departments of Biochemistry, Osmania and Kakatiya Universities. Circular Dichroism (CD) spectra was analyzed using web base Online K2D2 Software from NIN-Hyderabad. Far and near UV CD spectra were recorded using a spectropolarimeter (J-810, JASCO). Protein concentration used for far- and near-UV CD spectra was 1 and 1.5 mg/ml, respectively. RCP was micro filtered (0.4 size) in 20 mM sodium phosphate buffer (pH 7.4). The CD spectra were compared using K_2D_2 software. The method uses a self-organized map of spectra from195 nm to 250 nm proteins with known structure to deduce a map of protein secondary structure that is used to do the predictions. The CD spectra were compared using web based K2D2 software which is a publicly accessible web server at http://www. ogic.ca/projects/k2d2.

RESULTS AND DISCUSSION

K_2D_2 software is an online web server which is used to predict protein secondary structure from circular dichroism spectra. Different types of secondary structure produce characteristic spectra, which can be used to estimate its percentage content on the major secondary structure types. In this study, secondary structures of single poly peptide (219 amino acids), 29.2 kDa molecular weight of Riboflavin carrier proteins from hen, coot (white and yolk) and Owlet (white and yolk) were compared using K2D2 software and the results are discussed. We have observed the secondary structure of Riboflavin carrier protein content of hen, coot and spotted owlet eggs white and yolk. In hen and coot it was predicted to have same alpha (8.02 %) and beta stand percentages (22.14%) but in spotted owlet yolk variation in predicted alpha percentage (8.42%) and beta stand percentage (26.36%) was seen. The estimated measure of error was 0.32. The secondary structure relation is applied to estimate the alpha helix and beta strand percentages of a protein given its CD spectrum. K_2D_2 accepts a broader wavelength range for the input spectra. Figures (1, 2 and 3) and Table (1) gives the comparison between the secondary structures of both the yolk and white Riboflavin carrier proteins. Hen, coot and spotted owlet white RCP proteins showed similar molecular weight but their secondary structures differed. In spotted owlet yolk RCP molecular weight difference of 3kDa was observed. Secondary structure study showed alpha percentage (8.42%) and beta stand percentage (26.36%). The web based software K2D2 server is publicly accessible at http://www.ogic.ca/projects/k2d2/. It results accept as input a circular dichroism spectrum and give output of the secondary structure content in Riboflavin Carrier protein (alpha-helix and beta-strand) different percentages with an estimated measure of error.

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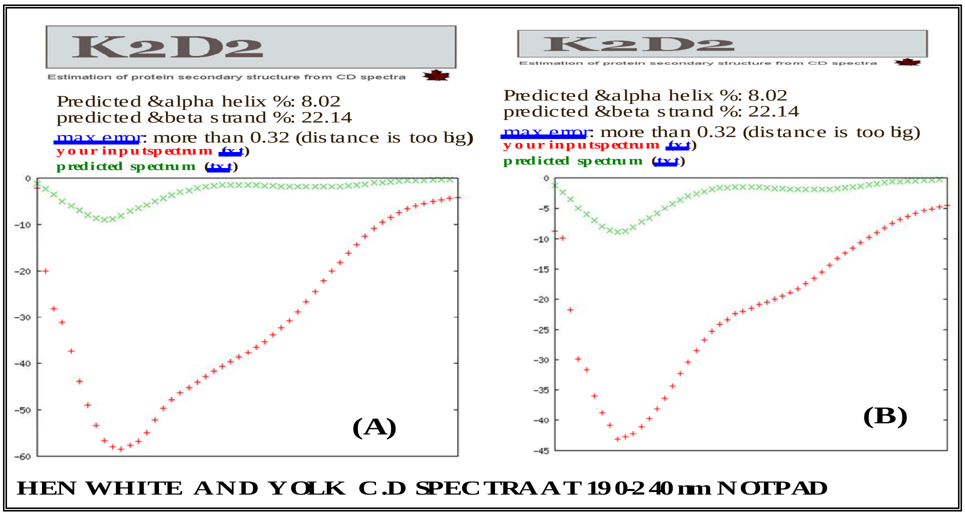

Figure: 1. K_2D_2 web server output spectrum for Hen egg White Riboflavin Carrier Protein and Hen Yolk Riboflavin Carrier Protein

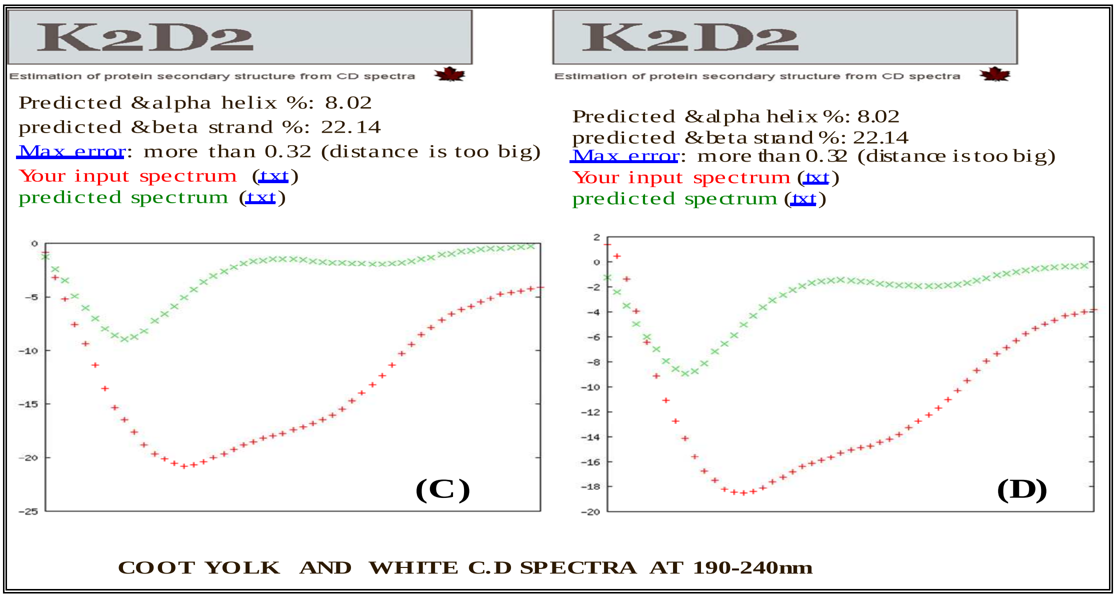

Figure: 2. K_2D_2 web server output spectrum for Coot egg yolk Riboflavin Carrier Protein and Coot White Riboflavin Carrier Protein

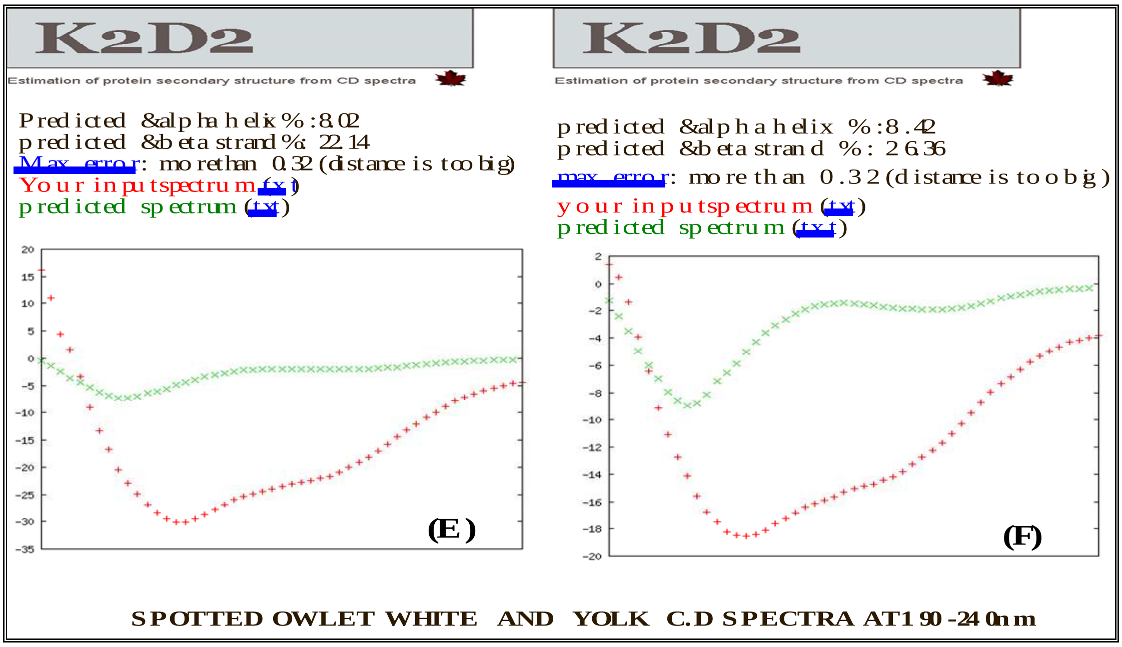

Figure: 3. K_2D_2 web server for Spotted Owlet egg Yolk Riboflavin Carrier Protein (E) and Spotted Owlet egg White Riboflavin Carrier Protein (F)

S.No	Riboflavin carrier protein (RfBP/ RCP) Isolated from different Eggs	Alpha helix (%) (208-222nm)	Beta stand (%)	Max error
A	Hen Egg White RCP	8.02	22.14	0.32
B	Hen Egg Yolk RCP	8.02	22.14	0.32
C	Coot Egg Yolk RCP	8.02	22.14	0.32
D	Coot Egg White RCP	8.02	22.14	0.32
E	Spotted Owlet Egg White RCP	8.02	22.14	0.32
F	Spotted Owlet Egg Yolk RCP	8.42	26.36	0.32

Table.1: Alpha and Beta (%) of Riboflavin Carrier protein (RCP) using K_2D_2 Software

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