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Research Article

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Assay of riboflavin carrier protein and its APO form using UV-visible spectrophotometry

Karunakar Rao Kudle and M. P. Pratap Rudra*

Department of Biochemistry, Osmania University, Hyderabad, India, T. S.

ABSTRACT

Riboflavin carrier protein from spotted owlet egg yolk has been isolated and it was assayed using spectrophotometry. The spectral intensity (1.687) of Rf solution showed small changes with a gradually increase in Riboflavin Carrier protein Binding capacity initially 10µl to 40µl and decreased binding capacity later on between 50 to 60 µl concentration. Isolated protein (RCP) binding affinity to Riboflavin was calculated at different concentrations and the results are communicated.

Key note: RCP = Riboflavin Carrier Protein, RF= Riboflavin, RCP-Riboflavin Complex

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INTRODUCTION

Vitamin binding proteins bind stoichimetrically and reversibly to vitamins with high affinity and receptor like specificity. Some of these vitamin carriers, those specific vitamins are constitutive while others specific to riboflavin. Upon physiological demand these binding proteins control the supply of active metabolite / coenzyme. These binding proteins are able to scavenge nutrients and the embryo from infection by microbes that require the ligands. The specific carrier proteins for vitamins such as vitamin A and vitamin D have been identified in normal serum in all vertebrates [1, 2, 3 and 4]. Proteins binding to water soluble vitamins such as Riboflavin binding/carrier proteins [5,6,7,8,9,10 and 11], vitamin B_{12} binding protein [12 and 13] and thiamin binding protein [14 and 15] have been demonstrated in the sera, egg white and yolk of the egg laying hens. Riboflavin binding protein **(**RfBP) is a phosphoglycoprotein, whose primary physiological function is to store riboflavin [16]. This carrier protein is essential for embryonic vitamin nutrition [17, 18, 19 and 20]. In the liver, RfBP is synthesized and is secreted into the blood stream, where it complexes with riboflavin. This complex is then deposited as part of the yolk in a developing oocyte. RCP was purified from spotted owlet (*Athene brama*) eggs. This was compared with hen (Gallus domesticus) RCP to understand the structural aspects, immunological characteristics, alpha(α) & beta (β) percentage in secondary structure of single poly peptide chain Riboflavin carrier protein(RCP) and isolated RCP against serum antibodies cytotoxicity activity study of the two avian species. Riboflavin carrier protein from hen and spotted owlet egg Riboflavin carrier protein have been isolated and their structures compared by SDS-PAGE here ours experiment observed spotted owlet yolk Riboflavin carrier protein molecular weight is 3kDa difference and secondary structure mapping study, Alpha (α), Beta (β) percentage in Riboflavin carrier protein is different [21]. No studies were conducted using spotted owlet Eggs before with a specific target against Riboflavin carrier Protein's of spotted owlet egg yolk, Binding Capacity and RCP-Riboflavin Complex (Quenched) studies.

EXPERIMENTAL SECTION

2.1 REAGENTS:

A. 1mg/ml Riboflavin Stock Solution (Prepare 500 ml in deionized water containing 1 ml of concentrated HCl using Riboflavin. **B.** 4.0 µg/ml Riboflavin Solution (Prepare 40 ml in deionized water using Reagent A. Prepare Fresh). **C.** 50 mM Sodium Acetate Buffer pH 5.5 at 25°C (Prepare 50 ml in deionized water using Glacial Acetic Acid and adjust pH to 5.5 with NaOH.) and **D.** Riboflavin Carrier Protein Solution (Prepare a solution containing 1 mg/ml).

3. SPECTRAL STUDIES:

3.1 UV-Spectra

The absorption spectrum of free Riboflavin was recorded using UV-Visible recording spectrophotometer (Lamda 25 Perkin Elmer). The Riboflavin solution contained 25 mg Riboflavin dissolved in 500 ml distilled water. 1ml of this standard solution was diluted to 5 ml with 0.05 M sodium phosphate buffer pH 7.4. The absorption spectra of the partially purified and purified RCP preparations were also recorded by diluting the protein suitably with the respective buffers or directly from the elutes of the column. Riboflavin (Rf) dissolved in buffer was transferred into one ml curette. Riboflavin was taken as blamk. The blank and the sample with Riboflavin Carrier protein was measured in the wavelength range between 190-600nm. In a 1.0 ml reaction mixture, 10 µl to 60 µl Riboflavin Carrier protein samples (0.0264 µg/10µl) gradually increased were used in this experiment. Biding capacity was expressed as (µg) Riboflavin bound per (µg) protein. Conditions in intensity Spectra, Binding Capacity, Light Path =1 Cm, T = 25°C, Ph = 5.5

Isolation of Riboflavin carrier protein (RCP) characteristic of Riboflavin-apoprotein forms (holoprotein) are the presented in the study. The free Riboflavin showed absorption maxima at 451nm. Binding of Riboflavin to the protein was checked using different aliquotes of protein (10µl= 0.0264 µg of`protein) resulting in the shift of the absorption peak from 451 nm to 447 nm and 448 nm at 10µl, 20 µl, 30 µl, 40 µl, 50 µl and 60 µl concentrations of protein respectively (peak intensity is increased but the wavelength was decreased).

RESULTS AND DISCUSSION

Table 1: Riboflavin binding of Riboflavin Carrier protein (Riboflavin-apoprotein complex)

<table>
<tr><td>S.No</td><td colspan="6">Riboflavin binding of Riboflavin Carrier protein (RCP)/ (Riboflavin-apoprotein complex)</td></tr>
<tr><td rowspan="2">1</td><td colspan="3" rowspan="2">Riboflavin (B_2)</td><td>Different Conc. (µl) of protein</td><td colspan="2">Wavelength range (190-600nm) and Absorbance values (Intensity Peaks)</td><td>After binding of protein estimation (µg)</td></tr>
<tr><td>10</td><td>447nm</td><td>1.689</td><td>0.0247</td></tr>
<tr><td rowspan="5">2</td><td>Riboflavin (1mg/1ml) (900 µl)</td><td>Wavelength Range (270-600nm)</td><td>Absorbance (Intensity)</td><td>20</td><td>448nm</td><td>1.739</td><td>0.0294</td></tr>
<tr><td rowspan="4">Riboflavin</td><td rowspan="4">451nm</td><td rowspan="4">1.687</td><td>30</td><td>448nm</td><td>1.764</td><td>0.0319</td></tr>
<tr><td>40</td><td>447nm</td><td>1.788</td><td>0.0355</td></tr>
<tr><td>50</td><td>448nm</td><td>1.770</td><td>0.0348</td></tr>
<tr><td>60</td><td>448nm</td><td>1.786</td><td>0.0350</td></tr>
</table>

In the present work, Riboflavin carrier protein (RCP) was purified for the first time from owlet egg yolk. Free riboflavin showed absorption maxima at 446 nm and 366 nm. However, in the holoprotein form (Riboflavin–apoprotein complex) the absorbance peak shifted to 451 nm, at the same time the absorbance band at 366 nm was shifted to 374 nm. Thus the spectra data were in complete agreement with the data reported for the flavoprotein and it was confirmed that the purified protein (Riboflavin carrier protein) were riboflavin–apoprotein complex (Figure.1). Riboflavin carrier protein elution profile on sephadex G-100 fraction by spectral O.D values at 280 nm & 455 nm [22 and 23]. Purified absorption spectrum. Further the visible absorption spectra revealed that the RCPs isolated had absorption maxima at 376 nm and 459 nm, characteristic of Riboflavin-apoprotein forms (holoprotein). The free Riboflavin showed absorption maxima at 451 nm. At the same time the absorption at 366 nm showed remarkable hypochromism without a shift of band position.

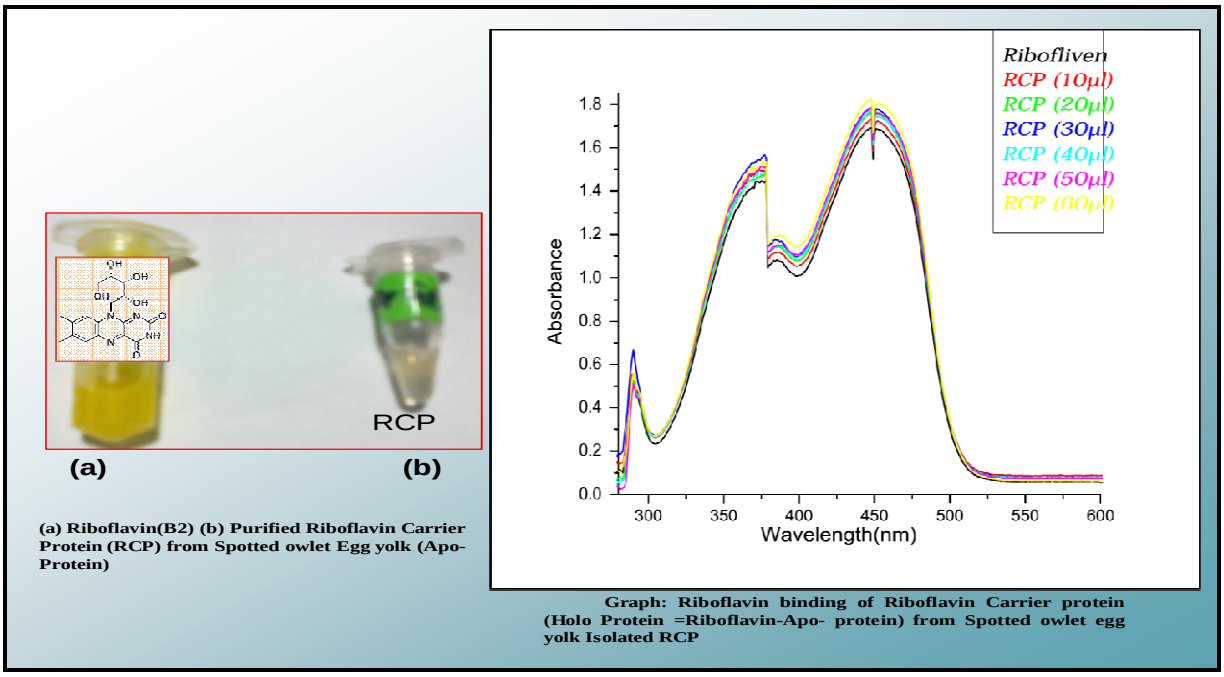

Figure 1: Riboflavin binding of Riboflavin Carrier protein (riboflavin-apoprotein complex)

However, in holo protein from spotted owlet egg yolk Riboflavin Carrier protein (RCP) absorption peak at 446nm shifted to 459 nm. In egg yolk RCP, absorption peak at 446 nm shifted to 457nm. Thus, the spectral data were in complete agreement with the data reported for the flavoprotein and it was confirmed that the purified protein was riboflavin apoprotein complex. The Visible absorption spectra revealed that the RCPs isolated absorption maxima at 376 nm and 459nm; characteristic of Riboflavin-apoprotein forms (holoprotein). Riboflavin carrier protein obtained from sephadex G-100 column was used for further studies. The free Riboflavin (Rf) showed absorption maxima at 451 nm. The absorption at 366 nm showed remarkable hypochromism without a shift of band position. However, isolation of Riboflavin carrier protein (RCP) characteristic of Riboflavin-apoprotein forms (holoprotein) study. The free Riboflavin showed absorption maxima at 451nm. Binding of Riboflavin to the protein was checked using different aliquotes of protein (10µl= 0.0264 µg of protein) resulting in the shift of the absorption peak from 451 nm to 447 nm and 448 nm 10µl to 60 µl concentration protein respectively (peak intensity is increased but the wavelength was decreased). The absorption spectra of the aqueous phase (pH 5.5) solution of Rf show a gradual increase in absorption at 451nm with a concomitant increase at around 385nm indicating the binding of Riboflavin Carrier protein. A decrease in the absorption of Riboflavin Carrier protein at 451 nm to 447nm and 448nm is due to the formation of Rf-RCP complex during the reaction. The spectra Intensity (1.687) of Rf solution show small changes with a gradually increase in Riboflavin Carrier protein Binding capacity 1.689 (0.0247µg), 1.739(0.0294µg), 1.764(0.0319µg), 1.788(0.0355µg), 1.770(0.0348µg) and 1.786 (0.0350µg) gradually increase 10µl to 40µl but 50µl and 60µl concentration decreased binding capacity. Riboflavin Carrier protein concentration compared to those observed in the present of Rf. Hence, we confirmed that the isolated protein (RCP) was binding to Riboflavin.

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REFERENCES

[1] Kanai, M., Raz, A., and Goodman, D.S., (**1968**). *J Clin. Invest*, 47: 2025-2044.
[2] Thomas, W.C., Morgan, H.G., Conner, T.B., Haddock, L., Bills, C.E., Howard, Jk, E., (**1959**). *J. Clin. Invest*, 38: 1078-1085

[3] Edelstein, S., Lawson., D.E.M. and Kodicek, K. (**1973**). J, 135: 417-426.
[4] Abe, T., Muto, Y. and Hosoya, N.J. (**1975**). *J. Lipid Res*. 16, 200-210.
[5] Rhodes, M.B., Bennett, N. and Feency R.E. (**1959**). *J. Bio chem.*Aug; 234(8):2054–2060.
[6] Ostrowski W, Skarzynski B, and Zak Z. (**1962**).. *Biochim biophys acta.May*. 21; 59:515–517.
[7] Karunakar Rao Kudle, M.P.Pratap Rudra, N.Veerababu and Ramchander Merugu. (**2012**). International Journal of Applied Biology and Pharmaceutical Technology. Volume -3, Dec- code: *IJABPT*, ISSN: 0976-4550.
[8] Madhukar Rao K, Prasad M.S.K. (**2011***). Int. J. of Appl. Biology*: 2; 27-29.
[9] Madhukar Rao K, Prasad M.S. K. (**2012a**). *Int. J. of Pharama. Sci*. R., 3; 494-496.
[10] Madhukar Rao K, Prasad M.S.K.(**2012b**). *Int. J. Appl. Biology*: 3; 351-354.
[11] Bindu Mary Rajan, Prasad M.S.K.(**2012**). *Iner. J. of Plant and Animal Scie.* 2; p 5-9.
[12] Grasbeck.R., (**1969**).. *Prog. Hematol*. 6: 233.
[13] Sonneborn D.W, Hansen H.J. (**1970**). *Science.* May 1; 168(3931):591–592.
[14] Naber, E.C, Cravens W.W, Baumann Ca, Bird Hr. (**1954**). *J. Nutr.* Dec 10; 54(4):579–591.
[15] Coates, M. E. (**1971**). (Eds J. Bell and B. M. Freeman) (London, New York: Academic Press) 1, 373.
[16] Rhodes, M.B., Bennett, N. and Feency R.E. (**1959**). *J. Bio chem.*Aug; 234(8):2054–2060.
[17] Maw, A.J.G. (**1954**). Poultry Sci.33, 216-17.
[18] Cowan, J.W., Boucher, R.U and Buss, E.G. (**1964**) and Cown, J.W., Boucher, R.U.and E.g. (1966) Poultry Sci.45, 538-41.
[19] Cowan, J.W, Boucher, R.U. and Buss, E.G (**1966**). *Poultry Sci*.45, 538-41.
[20] Winter WP, Buss EG, Clagett, C.O and Boucher, R.V. (**1967**). *Comp. Biochem.Physiol*. 22, 897-906.
[21] Karunakar Rao Kudle, Madhukar Rao Kudle, Ramchander Merugu and M. P. Pratap Rudra (**2015**). *Journal of Chemical and Pharmaceutical Research*, 7(11):288-291. ISSN: 0975-7384, CODEN (USA): JCPRC5.
[22] Karunakar Rao Kudle, M.P.Pratap Rudra (**2013**). *Int.J.Res.pharm.Science*.4 (4), 580-585.
[23] Karunakar Rao Kudle, M.P.Pratap Rudra, N.Veerababu and Ramchander Merugu.(**2012**) .*International Journal of Applied Biology and Pharmaceutical Technology*, volume-3, Issue-4,Oct-dec-**2012**,ISSN-0976-4550,Code-IJABPT