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In silico analysis of riboflavin carrier proteins from different *Avian species*

Abstract

A comparative in silico characterization of the Riboflavin carrier proteins (RCP) or Riboflavin binding proteins (RfBP) was carried out to analyze their physico-chemical, secondary structural and functional properties. The amino acid composition of Riboflavin binding/carrier proteins were obtained from biological databases. Molecular weights of all the proteins were around 27,000kD. pI value of *Cariama cristata* was the highest when compared to all other proteins. The instability index of all the proteins was more than 40 showing that all of them are probably not stable. Amino acid composition of vitamin binding proteins obtained from biological databases. The composition of serine and glutamic acid was high while low concentrations of Tryptophan, valine and glycine residues were seen when compared to other amino acids. Dominance of α-helices and random coils was observed from the secondary structural analysis of the proteins. SOSUI server analysis has shown that all the proteins are soluble in nature.

Keywords: RCP, *in silico*, physico chemical properties, secondary structure, sepharose column chromatography

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Introduction

Vitamin binding proteins bind reversibly to vitamins with high affinity and receptor like specificity in serum of vertebrates.[1–11] Riboflavin carrier proteins bind to riboflavin. RCP has been purified from many species. Vitamin binding proteins bind stoichiometrically and reversibly to vitamins with high affinity and receptor like specificity. Some of them are constitutive while some others are specific to riboflavin. These proteins supply coenzyme when there is physiological need and also regulate its supply. These binding proteins are able to scavenge nutrients and protect the embryo from infection. These specific carrier proteins like Riboflavin binding proteins, Thiamin binding proteins for vitamins have been identified in normal serum in all vertebrates.[1–4] Proteins binding to water soluble vitamins such as Riboflavin binding/carrier proteins,[5–11] vitamin B12 binding protein[12,13] and thiamin binding protein[14,15] have been demonstrated in the sera, egg white and yolk of the avian eggs. 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.[17–20] Sepharose column chromatography was used to purify Riboflavin binding protein (RfBP) from Hen (*Gallus gallus*) egg white and yolk.[8] *Aquila hastate* Riboflavin binding protein was purified by Kudle et al.[7] Emu (*Dromaius novaehollandiae*) Riboflavin-binding protein (RfBP) was purified from egg white by Bindu et al.[11] In the present study, a computational analysis of Riboflavin carrier proteins has been done and the results are discussed.

Materials and methods

UniProtKB/Swiss-Prot was used to retrieve the complete sequences of the Riboflavin carrier proteins. The computation of various physical and chemical parameters of the Riboflavin carrier proteins (aminoacids, positive charged residues, molecular weights, pI, negative extinction coefficient, aliphatic index, GRAVY instability index) was done using ExPASy's ProtParam tool. ExPASy's ProtScale tool was used to analyse hydrophobicity and transmembrane tendency.[21] SOPMA tool server was used to characterize the secondary structural features of Riboflavin carrier proteins.[22] The analysis of the Riboflavin carrier proteins motifs was done with the help of Motif Scan tool.[23] The SOSUI server prediction yielded the transmembrane regions of the Riboflavin carrier proteins.[24]

Results and discussion

Riboflavin carrier protein primary physiological function is to store riboflavin and transfer the vitamin to the embryo.[14–20] RfBP was isolated and purified from parrot eggs, peacock eggs.[25,26] Nikhath et al.,[25] purified RfBP for the first time from the yolk of parrot eggs using DEAE-Sephadex ion exchange chromatography followed by gel filtration on Sephadex G-100. Riboflavin binding protein (RfBP) from peacock eggs (*Pavo cristatus*) was purified by Rajendar et al.[26] Serum RfBP is synthesized in the liver after which complexes with riboflavin to form the holoprotein. If it is not complexed it is excreted by the kidney. The holoserum RfBP is removed from circulation by ovarian follicles and transported into the developing oocytes. Serum RfBP plays a protective role which is important in a riboflavin deficient diet. Holo-serum RfBP is transformed into holoyolk RfBP upon modification of its oligosaccharide moieties. The magnum of the oviduct synthesizes all egg white proteins and removes many proteins from the plasma as a source of its amino acid pool. After which it is catabolised with the subsequent release of riboflavin. This riboflavin is then captured by egg white RCP, synthesized by secretory cells of the magnum. The protein is conserved through evolution[12] and antibodies raised against this protein are capable of curtailing pregnancy. Karande et al.,[13] suggested that RfBP could be used for regulating fertility. Maehash et al.,[27] have reported the bitter inhibitory effect of Riboflavin-binding protein and hence it can also be used for reducing bitterness of foods. In our earlier works, we have purified the riboflavin binding proteins from different eggs.

Table 1 Physico chemical characteristics of riboflavin binding protein sequences

Species name	No. of amino acids	Molecular weight	PI	- Ve charged residues	+ Ve charged residues	Extinction coefficient	Instability index
Gallus gallus	238	27211.4	5.13	3.50E+01	23	46410	77.89
Dromaius	238	27343.9	5.25	34	25	53400	69.78
Merops nubicus	239	27390.7	5.58	32	26	54890	63.25
Charadrius vociferous	238	27346.8	5.52	33	25	53400	65.92
Cariama cristata	239	27563.2	7.37	31	32	53400	65.13
Nipponia nippon	240	27494.1	6.69	31	30	53400	64.32
Coturnix japonica	238	27237.4	5.36	34	23	44920	75.55

Table 2 Amino acid composition of Riboflavin binding protein sequences

Amino acids	*Gallus gallus*	*Dromaius*	*Merops nubicus*	*Charadrius vociferous*	*Cariama cristata*	*Nipponia Nnippon*
Ala	6.3	4.6	5.4	5	5	5
Arg	3.4	2.5	2.9	2.1	3.8	2.5
Asn	3.8	4.6	5.4	4.6	4.6	3.3
Asp	4.6	3.8	4.6	4.6	5.4	4.6
Cys	8	8	7.9	8	7.9	7.9
Gln	4.6	3.4	3.8	3.8	3.8	3.3
Glu	10.1	10.5	8.8	9.2	7.5	8.3
Gly	2.9	3.4	3.3	3.4	2.9	3.8
His	3.4	2.5	2.5	3.4	2.1	3.3
Ile	3.8	3.4	2.5	3.4	3.3	2.9
Leu	6.3	6.7	5.9	6.3	5.9	6.2
Lys	6.3	8	7.9	8.4	9.6	10
Met	3.4	3.8	3.3	3.8	3.8	3.8
Phe	3.4	3.4	3.3	3.4	3.3	3.3
Pro	3.4	3.4	3.3	3.4	3.3	3.3
Ser	13.4	13	13	12.2	13	13.3
Thr	4.2	4.2	4.2	4.6	4.6	5
Trp	2.5	2.9	2.9	2.9	2.9	2.9
Tyr	3.8	4.2	4.6	4.2	4.2	4.2
Val	2.5	3.8	4.2	3.4	2.9	2.9
Pyl	0	0	0	0	0	0
Sec	0	0	0	0	0	0

Table 3 Secondary structural analysis of Riboflavin binding proteins

Gallus gallus		*Dromaius*	*Merops nubicus*	*Charadrius vociferous*	*Cariama cristata*	*Nipponia nippon*
Alpha helix	50	42.02	38.49	42.44	40.59	35.42
310 helix	0	0	0	0	0	0
Pi helix	0	0	0	0	0	0
Beta bridge	0	0	0	0	0	0
Extended Strand	12.18	12.61	15.48	14.29	15.9	13.75

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Table Continued....

Gallus gallus		*Dromaius*	*Merops nubicus*	*Charadrius vociferous*	*Cariama cristata*	*Nipponia nippon*
Beta turn	6.3	6.72	8.79	7.98	9.21	7.5
Bend region	0	0	0	0	0	0
Random coil	31.51	38.66	37.24	35.29	34.31	43.33
Ambiguous state	0	0	0	0	0	0
Other state	0	0	0	0	0	0

Table 4 SOSUI server analysis of Riboflavin binding proteins

Nature	*Gallus gallus*	*Dromaius*	*Merops nubicus*	*Charadrius vociferous*	*Cariama cristata*	*Nipponia nippon*
Soluble/ Transmembrane	Soluble	Soluble	Soluble	Soluble	Soluble	Soluble

Conclusion

In this study the physicochemical properties of RCP proteins obtained from database are presented in Table 1. Negative charged aminoacids were more than positively charged aminoacids in the all the proteins compared (Table 1). Molecular weights of all the proteins were around 27,000kD. pI value of *Cariama cristata* was the highest when compared to all other proteins. The instability index of all the proteins was more than 40 showing that all of them are probably not stable. Amino acid composition of vitamin binding proteins obtained from biological databases is presented in Table 2. The composition of serine and glutamic acid was high while low concentrations of Tryptophan, valine and glycine residues were seen when compared to other aminoacids. From Table 3, dominance of α-helices and random coils was observed from the secondary structural analysis of the proteins. SOSUI server analysis Table 4 has shown that all the proteins are soluble in nature.

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None.

Conflict of interest

The author declares no conflict of interest.

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