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/carryer proteins were obtained from biological databases. Molecular weights of all the proteins were around 27,000D. pI value of Carinama cristata was the highest when compared to all other proteins. The instability index of all the proteins was more than 50% 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

Introduction

Vitamin binding proteins bind reversibly to vitamins with high affinity and receptor like specificity in serum of vertebrates. 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 from different eggs. Vitamin binding proteins from different Avian species have been isolated and purified from parrot eggs, peacock eggs, koklass pheasant eggs, Nikkhath et al., purified RibfBP for the first time from the egg white 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 Rajender et al. Serum RfBP is synthesised 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 synthesises 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.

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, pl, 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. SOPMA tool server was used to characterize the secondary structural features of Riboflavin carrier proteins. The analysis of the Riboflavin carrier proteins motifs was done with the help of Motif Scan tool. The SOSUI server prediction yielded the transmembrane regions of the Riboflavin carrier proteins.

Results and discussion

Riboflavin carrier protein primary physiological function is to store riboflavin and transfer the vitamin to the embryo. Riboflavin binding protein was purified by Kudle et al. from Hen (Gallus gallus) egg white and yolk. Agila hastate Riboflavin binding protein was purified by Kudle et al. Emu (Dromaius novaehollandiae) Riboflavin-binding protein (RfBP) was purified from egg white by Bindu et al. In the present study, a computational analysis of Riboflavin carrier proteins has been done and the results are discussed.
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 nippon |
|-------------|---------------|----------|----------------|-----------------------|------------------|-----------------|
| 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           | 5.4      | 4.6            | 4.6                   | 5.4              | 4.6             |
| Asp         | 4.6           | 3.8      | 4.6            | 5.4                   | 4.6              | 4.6             |
| Cys         | 8             | 8        | 7.9            | 8                     | 7.9              | 7.9             |
| Gln         | 4.6           | 3.4      | 3.8            | 3.8                   | 3.8              | 3.8             |
| 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      | 3.4            | 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....

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

Table 4 SOSUI server analysis of Riboflavin binding proteins

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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