# *In silico* Characterization of Retinal *S*-antigen and Retinol Binding Protein-3: Target against Eales' Disease

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***Abstract:*** *In current study two important proteins of Eales' disease, i.e. Retinal S-antigen (RSAG) (P10523) and Retinol binding protein-3 (RBP-3) (P10745), retrieved from Swiss prot database, are analysed and characterized through in silico tools. Characterization is carried out in terms of molecular weight, atomic composition, isoelectric point, extinction coefficient, aliphatic index and instability index. Primary structure analysis of target proteins showed that most of the amino acids are hydrophobic in nature which was evident due to the high content of non-polar residues. Thermal stability, which is a notification regarding the flexibility in the structure of protein, is higher here, i.e. 88.27 for RSAG and 100.31 of RBP-3 suggesting their stability in a wide range of temperature. Secondary structure analysis of RBP-3 and RSAG reveals that RBP-3 mostly have alpha helices while RSAG have mixed secondary structures, i.e. the alpha helices, extended strands and random coils which is suggestive about the high structural conservity of protein. This evolutionary conservity makes RSAG a better target against Eales' disorder. Determination of phosphorylation as well as signal peptide cleavage sites is another integral part of in silico characterization, as these determinations confirms about the functional aspect of protein.*

***Keywords:*** *Eales' disease, Retinal S-antigen, Retinol binding protein-3, Computational analysis, Inflammation, Haemorrhage.*

## Introduction

Eales' disease is one among the ocular disorders, where the etiopathology is largely unknown, thus hampers the therapeutic interventions. The treatment is still illusive and symptomatic only. It is manifested by retinal periphlebitis, peripheral retinal ischemia, haemorrhage, the stage of inflammation and the stage of proliferation. Retinal *S*-antigen (RSAG) and Interphotoreceptor Retinol binding protein (RBP-3) play a significant role in the etiopathology of this condition. The key role of RSAG and RBP-3 has been documented well

in the etiopathogenesis of this disease. This RSAG protein has already been isolated and purified from the whole retina as a soluble protein of 48-kD of rod outer segments. The purified protein shows specific binding to photo excited rhodopsin and found to involve in the quenching of light-induced guanosine 3′,5′-monophosphate-phosphodiesterase activity [1, 2, 7-9, 20, 22-24, 26, 32, 33]. In order to demonstrate the specific involvement of RSAG and RBP-3 in the pathogenesis of Eales' disease, the studies have been carried out for lymphocyte proliferative responses, uveitopathogenic peptides (peptide M and peptide G), yeast histone H3 peptide and uveitopathogenic fragments of Interphotoreceptor retinoid binding protein (IRBP; R16) [21].

Bioinformatics is an interdisciplinary research area work which acts as an interface between biological and computational science to solve ample of important issues including structural and functional analysis of protein. Role of bioinformatics is well established even in ocular biology.

This work signifies the potential contribution of *in silico* based protein specific study in Eales' disease [18, 29, 34]. Physicochemical characterization of proteins give a better idea about the properties like molecular weight, atomic composition, isoelectric point, extinction coefficient, aliphatic index (AI), grand average hydrophathy (GRAVY) and instability index [4, 6, 15, 16, 28]. All these parameters play an important role in deciphering the properties of protein under analysis. The current course of work comparative illustration of the physicochemical characterization of both the proteins is narrated through manual and computational programs. Prediction of secondary structure of protein is another important parameter in its structural and functional analysis. Determination of various phosphorylation and signal peptidal cleavage sites help in exploring their functional aspects as phosphorylation and dephosphorylation switches "on" and "off" the various bio-mechanisms in many enzymes and receptors. In current course of work, the above mentioned proteins were characterized as well as their secondary structure was predicted for better structural and functional understanding of the selected proteins.

## Materials and methods

### *Protein sequence identification*

Protein sequences of RSAG and RBP-3 were retrieved from the manually curated public protein database, i.e. UniProt, through their accession number P10523 and P10745 respectively [31]. The sequences were retrieved in FASTA format and further used for primary and secondary level structural analysis.

### *Tools and servers*

The amino acid composition of corresponding protein sequences was calculated using the tool ProtParam from ExPasy (Expert protein analysis system) [10]. Percentage of hydrophobic and hydrophilic residues was calculated manually on the basis of their nature from the results obtained after primary structure analysis. The physico-chemical parameters, i.e. theoretical isoelectric point (pI), molecular weight, total number of positive and negative residues, extinction coefficient, half-life, instability index, AI and GRAVY were computed using the Expasy's ProtParam prediction server. Secondary structure prediction was done by using SOPMA server [11, 27, 30]. The NetPhos-2.0 server was used for studying potential phosphorylation sites of protein [3]. Further Signal P-4.1 server was used to denote the presence and location of signal peptide cleavage sites in given sequences [19].

## Results and discussion

Primary structure analysis in terms of *in silico* characterization reveals that RSAG is more hydrophobic than RBP-3 evident from percentage calculation of non-polar amino acid content in RSAG and RBP-3, i.e. 47.1 and 52.5, respectively (Table 1). The computed pI values of RSAG and RBP-3 are 6.13 and 4.98 respectively, which are indicative towards the information that these proteins are acidic and will be more stable and compact at their respective pH. The computed pI can be useful in developing buffer systems for purification through isoelectric focusing method. Extinction coefficient of RSAG and RBP-3 at 280 nm is 26485 $M^{-1} \cdot cm^{-1} \cdot s$ and 136640 $M^{-1} \cdot cm^{-1} \cdot s$ with respect to the concentration of cystine (Cys), tryptophan (Trp) and tyrosin (Tyr). The high extinction coefficient of these proteins indicates presence of high concentration of Cys, Trp and Tyr which illustrates that these proteins can easily be analyzed using UV spectral methods and can also be helpful in docking studies [12]. The biocomputed half-life of these proteins is 30 hours.

Table 1. Amino acid composition of Retinal *S*-antigen and Retinol binding protein-3 proteins

| | **Retinal *S*-antigen (P10523)** | | **Retinol binding protein-3 (P10745)** | |
|---|---|---|---|---|
| **Amino acid** | **No. of amino acid** | **Composition (in %)** | **No. of amino acid** | **Composition (in %)** |
| Ala | 27 | 6.7 | 109 | 8.9 |
| Arg | 16 | 4.0 | 54 | 4.4 |
| Asn | 14 | 3.5 | 22 | 1.8 |
| Asp | 26 | 6.4 | 59 | 4.8 |
| Cys | 3 | 0.7 | 7 | 0.6 |
| Gln | 15 | 3.7 | 55 | 4.5 |
| Glu | 29 | 7.2 | 84 | 6.8 |
| Gly | 20 | 4.9 | 95 | 7.7 |
| His | 8 | 2.0 | 37 | 3.0 |
| Ile | 18 | 4.4 | 51 | 4.1 |
| Leu | 37 | 9.1 | 154 | 12.5 |
| Lys | 35 | 8.6 | 30 | 2.4 |
| Met | 6 | 1.5 | 27 | 2.2 |
| Phe | 16 | 4.0 | 33 | 2.7 |
| Pro | 26 | 6.4 | 82 | 6.7 |
| Ser | 28 | 6.9 | 91 | 7.4 |
| Thr | 26 | 6.4 | 81 | 6.6 |
| Trp | 1 | 0.2 | 14 | 1.1 |
| Tyr | 14 | 3.5 | 36 | 2.9 |
| Val | 40 | 9.9 | 109 | 8.9 |

On the basis of instability index Expasy's ProtParam classifies P10745 as unstable (instability index = 43.45) and P10523 as stable (instability index = 34.90) [13]. The AI determines the thermal stability of globular proteins on the basis of presence of alanine, valine and leucine. Lower thermal stability of proteins indicates more flexible

structure, but in current study it is high enough (88.27 for RSAG and 100.31 of RBP-3) to suggest their stability for a wide range of temperature [14]. GRAVY value for a peptide or protein is calculated as the sum total of hydrophathy values of all the amino acids, divided by the number of residues in the sequence. The very low GRAVY index of P10523 infers that this protein could result in a better interaction with water (Table 2) [17]. Expansion of the study in terms of secondary structure predicted with the help of program SOPMA, infers that the RBP-3 mostly have alpha helices while RSAG have mixed secondary structures, i.e. alpha helices, extended strands and random coils. This prediction suggests that studied proteins are structural in nature, such as proteins are rich in secondary confirmation [25, 35] (Fig. 1). Phosphorylation is the addition of a phosphate ($PO4^{3-}$) group to a protein or other organic molecule which plays a significant role in a wide range of cellular processes. Determination of protein phosphorylation sites is usually the initial step in the explanation of any regulatory mechanism and its description is the prerequisite for the functional analysis of phosphorylation by mutational analysis. Information regarding the phosphorylation site is also essential for the purification of the upstream kinase that phosphorylates the defined sites. Among the three complementary approaches for determination of phosphorylation sites, i.e. bioinformatics, genetics and the biochemical approach. Bioinformatics approach is currently more in demand and is much more recognized in recent research trends. Phosphorylation is an important characteristic feature which is basically dependent on the presence of serine (Ser), threonine (Thr) and tyrosine residues in eukaryotic proteins [5]. The predicted phosphorylation sites in RSAG were Ser: 10, Thr: 7, Tyr: 5 and for RBP-3, they were Ser: 40, Thr: 20, Tyr: 13 (Fig. 2). Cleavage sites are specific peptide sequences, or more often, peptide motifs, where site-specific proteases cleave or cut the protein. These specific sites can be used to cleave off an affinity tag thereby restoring the natural protein sequence or to inactivate a protein. Fig. 3 illustrates the signal peptide cleavage sites for RBP-3 and RSAG through Signal P4.1 server. Results are illustrated in terms of C (raw cleavage site score), S (signal peptide score) and Y (combined cleavage site score) scores. For RBP-3 C, S and Y scores are 0.457, 0.635, 0.958 and for RSAG the scores are 0.122, 0.106, and 0.117 respectively.

Table 2. Physicochemical parameters of Retinal *S*-antigen and Retinol binding protein-3 [14]

| | **Retinal *S*-antigen** | **Retinol binding protein-3** |
|---|---|---|
| Protein accession No. | P10523 | P10745 |
| Sequence length | 405 | 1247 |
| Molecular weight | 45119.5 | 135362.6 |
| Theoretical pI | 6.13 | 4.98 |
| -R | 55 | 144 |
| +R | 51 | 85 |
| Extinction coefficients | 26485 | 136640 |
| Instability index | 34.90 | 43.45 |
| Aliphatic index | 88.27 | 100.31 |
| Grand average of hydropathicity | -0.335 | 0.041 |

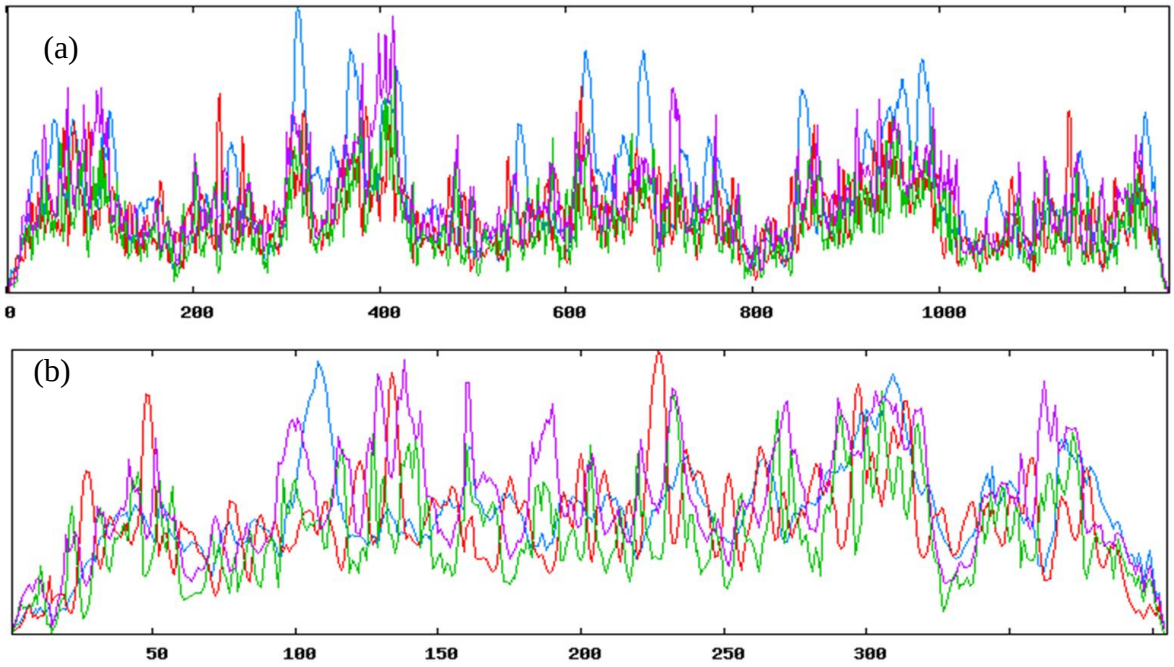


Fig. 1 Results of SOPMA analysis
(a) Retinol binding protein-3; (b) Retinal *S*-antigen.
Blue, red, green and light yellow colour lines shows percentage
of alpha helices, extended strand, beta turn and random coil.

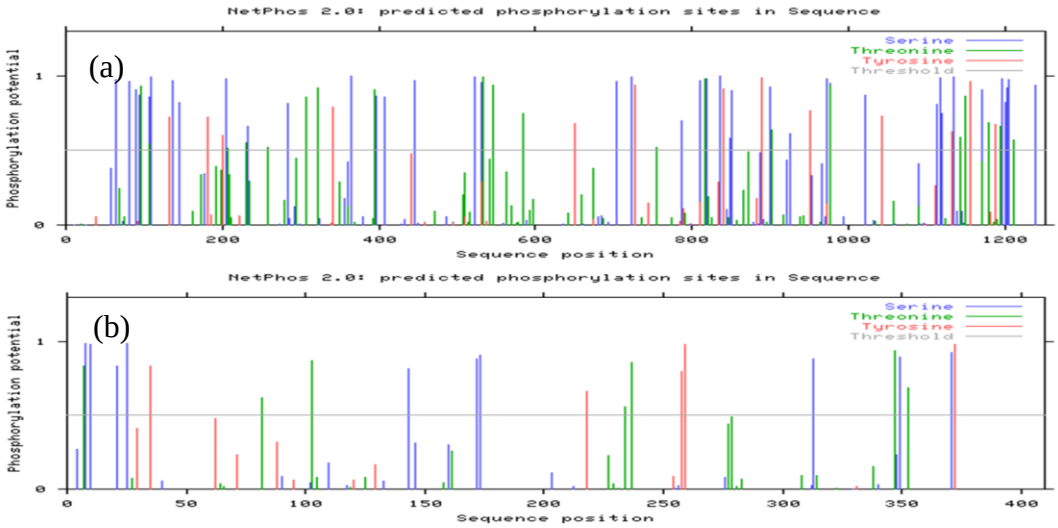


Fig. 2 Results of NetPhos analysis
(a) Retinol binding protein-3; (b) Retinal S-antigen.
Blue, green and red line shows the total count of serine;
threonine and tyrosine residues participated in phosphorylation.

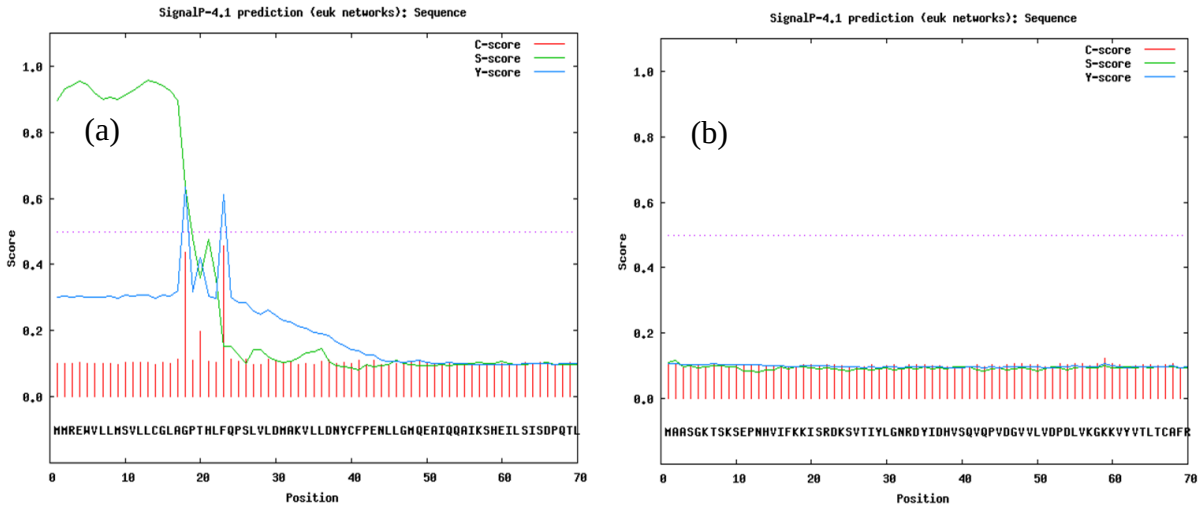


Fig. 3 Results of Signal-P analysis
(a) Retinol binding protein-3; (b) Retinal *S*-antigen displayed in terms of C, S and Y score

## Conclusion

*In silico* characterization is important in deciphering the important physical and chemical properties along with the prediction of basic conformation of proteins in their secondary structure. This study signifies the detail interpretations of physico chemical studied parameters. These basic to advanced features of proteins can give a leading idea about their structural and functional aspects. Furthermore, comparison of results during *in silico* characterization of more than one protein gives very clear cut comparative results and aspects in terms of target identification. In current course of work by comparing the results of secondary structure prediction of selected proteins, it can be concluded that RSAG is more stable than RBP-3 as it is rich in super secondary structures which are actually the motif regions and are highly conserved during the evolutionary processes. This may be the explanation for the stability of RSAG. RBP-3 is a binding protein, so it may need some modifications to get into proper stable form. Determination of phosphorylation as well as signal peptide cleavage sites explores functional aspects of protein in more depth including stability index of protein. Therefore, it can be concluded from relevant findings that for the etiopathology purpose as well as for future therapeutic aspects related to Eales' disease, it will be better to target protein RSAG in comparison with RBP-3.

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