RESEARCH ARTICLE

In silico structural characterization of Monooxygenase and dioxygenase enzymes from *Danio rerio*

Chittaranjan Baruah[1,*] and Papari Devi[2]

[1]Department of Zoology, Darrang College, Tezpur – 784001, Assam, India
[2]Department of Zoology, Kaliabor College, Kuwarital-782137, Assam, India
*Corresponding author: chittaranjan_21@yahoo.co.in

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The paper is dedicated with deep reverence to Late Professor UC Goswami, Former Professor and Head of Department of Zoology, Gauhati University, Assam, India who inspired the authors for molecular and in-silico analysis of Retinoids and Carotinoids.

ABSTRACT

Freshwater fish having both retind and dehydretind showed the capabilities of clearing the provitamin A-Status carotenoids into both forms of Vitamin A, either through central or terminal cleavage. The involvement of monooxygenase and dioxygenase enzymes system has been identified. The present manuscript is presenting an *in silico* analysis that has been performed for molecular modeling and functional annotation of Monoxygenase and Dioxygenase enzymes from *Danio rerio* using sequence data extracted from publicly available database. The functional annotations have been performed using several publicly available Bioinformatics tools and databases. The study represents the application of comparative modeling method for 3D structure prediction. The functional annotations have been performed using several publicly available Bioinformatics tools and databases.

Keywords: Beta-carotene, Vitamin A, Homology modelling, phylogeny, ortholog, paralog

INTRODUCTION

Retinoids play an essential role in vision, cell differentiation, embryonic development, membrane and skin protection and are crucial for the health of mammals and other vertebrates. Retinoids are obtained from provitamin A carotenoids, like β-carotene, through the oxidative cleavage by β-carotene 15, 15′ monooxygenase enzyme. It belongs to an extended family of dioxygenases which include the plant neoxanthin cleavage enzymes, the bacterial lignostilbene dioxygenases, and the vertebrate protein RPE65. Members of this family interact with carotenoids and other polyenes (Redmond *et al*., 2001).

The mechanism of the formation of Vitamin A has been studied since 1930(March, 1930, 1957) and several workers has identified the involvent of both monooxygenase and dioxygenase enzyme catalyzing the clearing process of B-carotene and others either centrally or terminally stepwise following B- oxidation (Barua and Goswami, 1977). We have reported the

metabolism of B-carotene, lutein, astaxanthin and cryptoxanthin in both retinoid-rich as well as dehydroretind-rich fish. Considering the involvement of dioxygenase and mono-oxygenase system in clearing the caronotenoids molecules in the above mentioned pesitien, the structure of the enzymes has been elucidated as described in Zebra fish, *Danio rerio*. D. rerio is widely distriunted freshwater fish of the cyprinidae, which is rich in retind (75%) and dehydroretind (25%) (Barua *et al*., 1973; Goswami and Baruah, 1981).

Although there is an availability of sequence information for monooxygenase and dioxygenase enzymes from *Danio rerio,* yet there is no structural information and functional annotation available. Therefore, the biochemistry and molecular mechanism of their functions are still not very well understood. The present study was carried out to predict the 3D folding pattern (Zemla *et al.*, 1999), sequence based functional annotation of monooxygenase and dioxygenase enzymes from *Danio rerio* and their sequence variation to identify their structural and evolutionary properties in order to characterize the role of the two enzyme in fish model.

MATERIALS AND METHODS

A. Acquisition and alignment of sequences

The sequences of *Danio rerio* monooxygenase and dioxygenase enzymes were acquired from the UniProtKB (Accession no. Q1RLW1 and Q7ZTS0 respectively). The significance of the BLAST results was assessed by expect values (e-value) generated by BLAST family of search algorithm (Altschul *et al.*, 1991). ClustalW analysis software available online from EBI was used to compare sequence alignment of monooxygenase and dioxygenase enzymes (www.ebi.ac.uk/clustalw/). Default settings were used except that a gap extension setting of 3 was used. ClustalW results were illustrated using Boxshade v3.21(http://www.ch.embnet.org/software/BOX_form.html).

B. Three-dimensional structure prediction

For Comparative (Homology) modeling,the 3D coordinates of of pdb ID 2BIW Chain A (Crystal structure of apocarotenoid cleavage oxygenase from synechocystis, native enzyme with) have been choosed as the suitable template which shows E-value of 1e-05 with 23.8 % identity and 520 overlap in the BLAST result. The target-template alignment and model building were conducted manually by using Modeller program (Marti-Renom *et al.*, 2000). The *ab-initio* loop modeling was conducted by MODLOOP server. The final 3D structures with all the coordinates for both the targets were obtained by optimization of a molecular probability density function (pdf) of Modeller as well as MODLOOP (Eswar *et al.*, 2006). The 3D structures *were* evaluated (Giorgetti *et al.*, 2005) by ProCheck (Laskowski *et al.*, 2003). After fruitful verification of the coordinate files, the structures were successfully deposited to PMDB for the access to the scientific community (Tiziana *et al.*, 2006). All the graphic presentations of the 3D structures were prepared using Chimera (Pettersen *et al.*, 2004).

C. Sequence based functional annotation

The sequence based functional annotation have been carried out for dioxygenase and monooxygenase enzymes of 536 sequences from 204 different species in the sequence database, using Pfam (*pfam.sanger.ac.uk/*), GO (www.geneontology.org*/).* KEGG database (www.genome.jp/kegg/). Orthologs paralogs have been identified and details given in the phyologenetics analysis.

a. Beta-carotene 15, 15-monooxygenase 2 **KEEG ID: dre: dre:84039.**

Gene ontology shows the monooxygenase activity through catalysis of the incorporation of one atom from molecular oxygen into a compound and the reduction of the other atom of oxygen to water.

b.Beta-carotene 15, 15-dioxygenase 2 : **KEEG ID: dre:678554**

Oxidoreductase activity, acting on single donors with incorporation of molecular oxygen, incorporation of two atoms of oxygen. (From Gene ontology database.)

RESULTS AND DISCUSSION

The predicted 3D Structures

The models of monooxygenase and dioxygenase enzymes from *Danio rerio* have been deposited to PMDB with the assignment unique PMDB IDs PM0075185 and PM0075184 respectively (Figures 1,2 &3) for the coordinate entry. The structure of Monooxygenase has 371 H-bonds, 6 helices, 51 strands

and 68 turms while the structure of Dioxygenase has 403 H-bonds, 6 helices, 55 strands and 62 turns. Procheck verification proved that the models are of good quality as judged by Ramachandran Plot (Ramachandran & Sasisekharan, 1968).

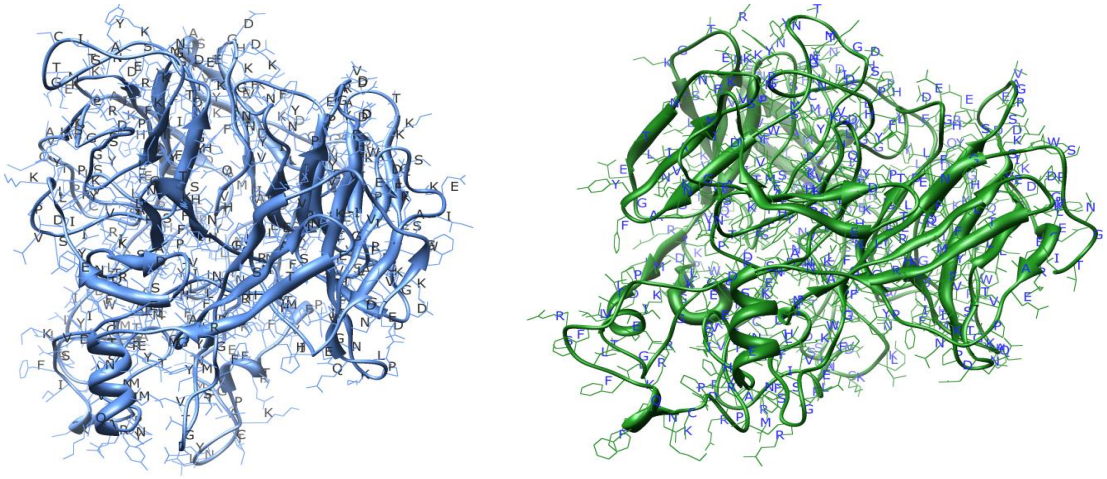

Figure 1. The Theoretical models. **A.** Beta-carotene 15, 15'-monooxygenase 1 (PM0075185) and **B.** Beta-carotene 15, 15-dioxygenase 2 (PM0075184), displayed by UCSF Chimera.

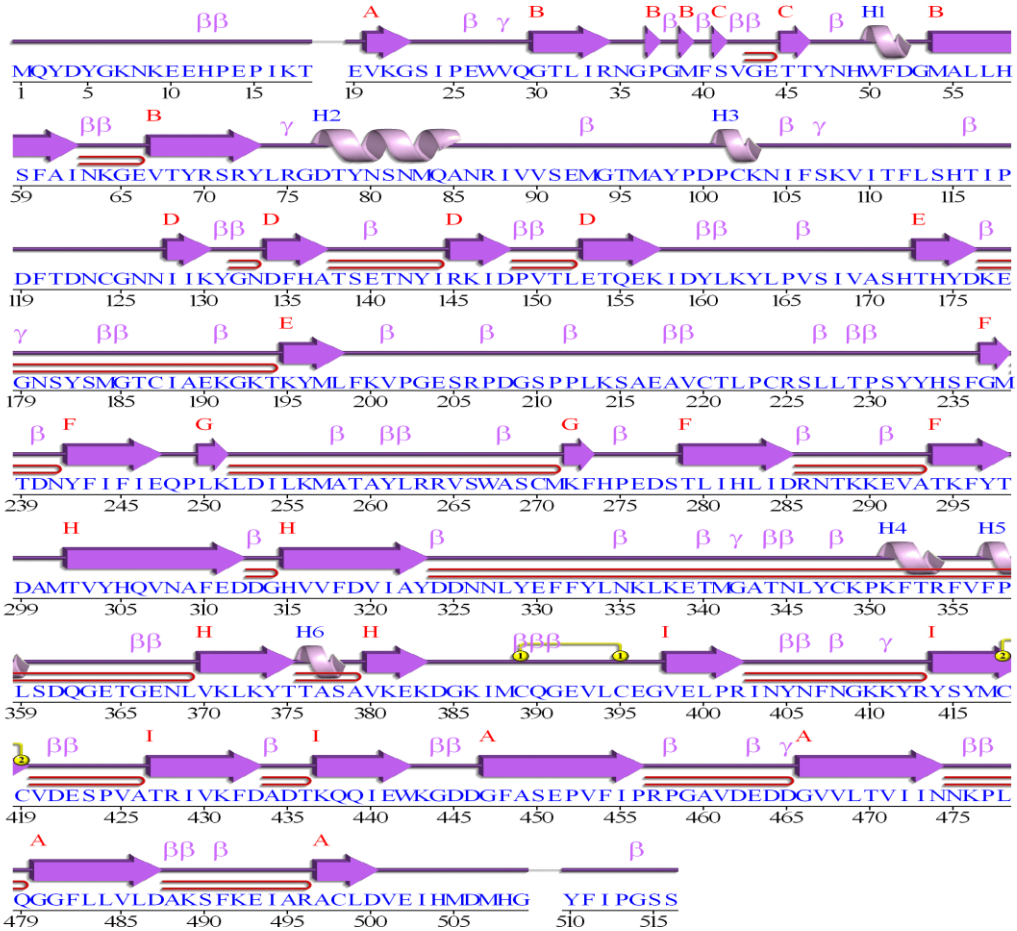

Figure 2. The secondary structure information of Beta-carotene 15, 15'-monooxygenase 1 (PM0075185).

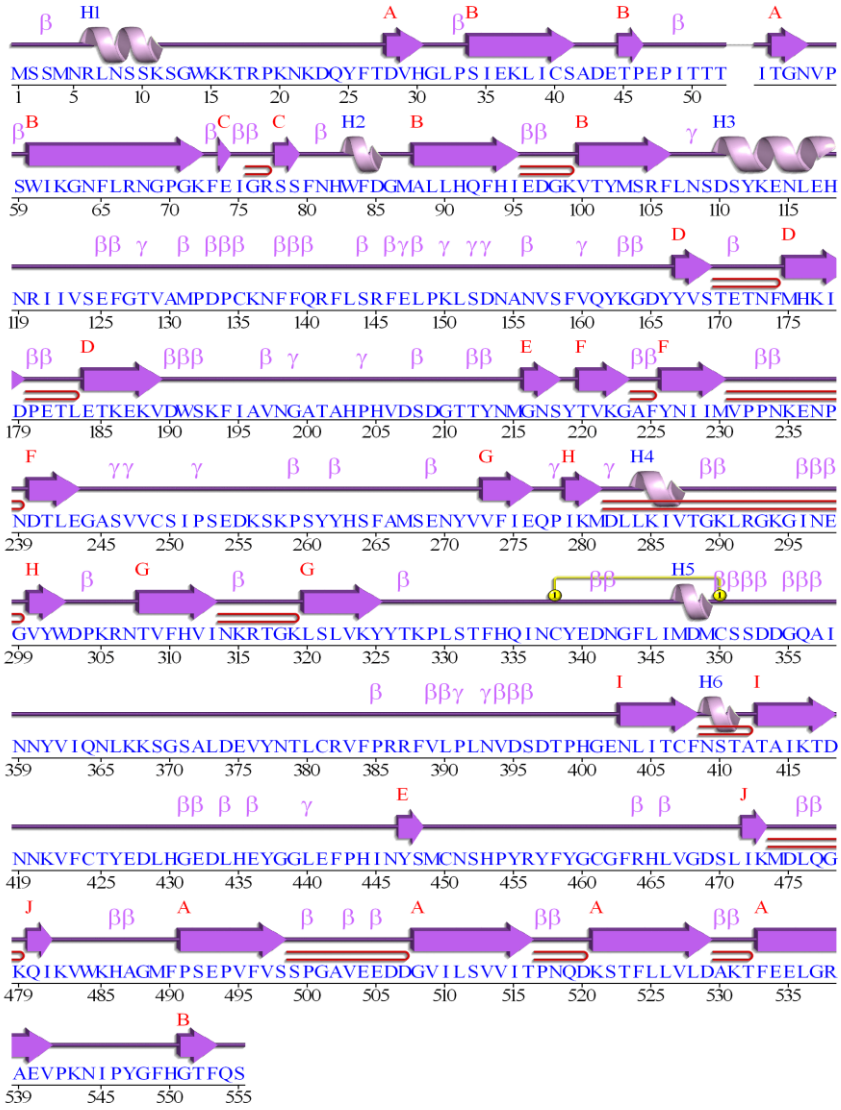

Figure 3. The secondary structure information of Beta-carotene 15, 15-dioxygenase 2 (PM0075184).

Functional annotation

```
KEGG Orthology (KO) [BR:dre00001]
  01100 Metabolism
    01190 Metabolism of Cofactors and Vitamins
      00830 Retinol metabolism [PATH:dre00830]
        84039 bcmo1; beta-carotene 15,15'-monooxygenase 1 ; K00515 beta-carotene 15,15'-monooxygenase
              [EC:1.14.99.36]

Enzymes [BR:dre01000]
  1. Oxidoreductases
    1.14  Acting on paired donors, with O2 as oxidant and incorporation or reduction of
              oxygen. The oxygen incorporated need not be derived from O2
      1.14.99  Miscellaneous
        1.14.99.36  beta-carotene 15,15'-monooxygenase
          84039 bcmo1; beta-carotene 15,15'-monooxygenase 1 ; K00515 beta-carotene 15,15'-monooxygenase
              [EC:1.14.99.36]
```

a. **Beta-carotene 15,15'-monooxygenase 1**

Catalysis of the incorporation of one atom from molecular oxygen into a compound and the reduction of the other atom of oxygen to water. (From Gene ontology database.).

b. ***Beta-carotene 15, 15-dioxygenase 2***

Oxidoreductase activity, acting on single donors with incorporation of molecular oxygen, incorporation of two atoms of oxygen. (From Gene ontology database; Figures 4 & 5).

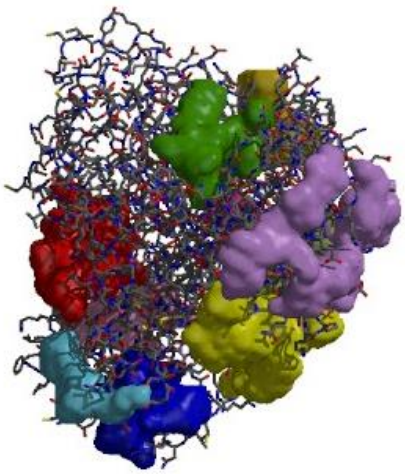

Fig. 4

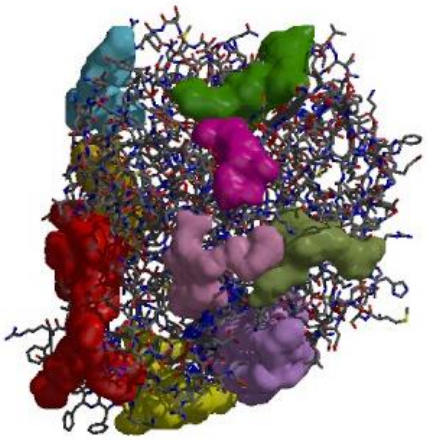

Fig. 5

Figure 4. Clefts and cavities in protein structure, *Beta-carotene 15,15'-monooxygenase 1* the colours corresponding to the entries in the table below.

Figure 5. Clefts and cavities in protein structure, *Beta-carotene 15, 15-dioxygenase 2* the colours corresponding to the entries in the table below

Fig. 4

* **Residue-type colouring:**									
Positive	**Negative**	**Neutral**	**Aliphatic**	**Aromatic**	**Pro & Gly**	**Cysteine**			
H,K,R	D,E	S,T,N,Q	A,V,L,I,M	F,Y,W	P,G	C			
* **Colouring by residue-conservation score:**									
blue	purple	skyblue	cyan	grey	green	yellow	orange	pink	Red
0.0-0.1	0.1-0.2	0.2-0.3	0.3-0.4	0.4-0.5	0.5-0.6	0.6-0.7	0.7-0.9	0.8-0.9	0.9-1.0

Fig. 5

Residue-type colouring:									
Positive	**Negative**	**Neutral**	**Aliphatic**	**Aromatic**	**Pro & Gly**	**Cysteine**			
H,K,R	D,E	S,T,N,Q	A,V,L,I,M	F,Y,W	P,G	C			
** **Colouring by residue-conservation score:**									
blue	purple	skyblue	cyan	grey	green	yellow	orange	pink	Red
0.0-0.1	0.1-0.2	0.2-0.3	0.3-0.4	0.4-0.5	0.5-0.6	0.6-0.7	0.7-0.9	0.8-0.9	0.9-1.0

KEEG ID: dre:678554

```
Enzymes [BR:dre01000]
  1. Oxidoreductases
    1.14  Acting on paired donors, with O2 as oxidant and incorporation or reduction of
                oxygen. The oxygen incorporated need not be derived from O2
      1.14.99  Miscellaneous
        1.14.99.-
          678554 bco2a; beta-carotene oxygenase 2a ; K10252 beta,beta-carotene 9',10'-dioxygenase
                  [EC:1.14.99.-]
```

Interpro entry IPR004294

Carotenoids such as beta-carotene, lycopene, lutein and beta-cryptoxanthine are produced in plants and certain bacteria, algae and fungi, where they function as accessory photosynthetic pigments and as scavengers of oxygen radicals for photoprotection. They are also essential dietary nutrients in animals. Carotenoid oxygenases cleave a variety of carotenoids into a range of biologically important products, including apocarotenoids in plants that function as hormones, pigments, flavours, floral scents and defence compounds, and retinoids in animals that function as vitamins, visual pigments and signalling molecules PUBMED:14704328. Examples of carotenoid oxygenases include:

- Beta-carotene-15,15'-monooxygenase (BCDO1;) from animals, which cleaves beta-carotene symmetrically at the central double bond to yield two molecules of retinal PUBMED:14704328.
- Beta-carotene-9',10'-dioxygenase (BCDO2) from animals, which cleaves beta-carotene asymmetrically to apo-10'-beta-carotenal and beta-ionone, the latter being converted to retinoic acid. Lycopene is also oxidatively cleaved PUBMED:14704328.

- 9-cis-epoxycarotenoid dioxygenase from plants, which cleaves 9-cis xanthophylls to xanthoxin, a precursor of the hormone abscisic acid PUBMED:12834401.
- Apocarotenoid-15,15'-oxygenase from bacteria and cyanobacteria, which converts beta-apocarotenals rather than beta-carotene into retinal. This protein has a seven-bladed beta-propeller structure with four hisitidines that hold the iron active centre PUBMED:15821095.
- Retinal pigment RPE65 from animals, which in its soluble form binds all-trans retinol, and in its membrane-bound form binds all-trans retinyl esters. RPE65 is important for the production of 11-cis retinal during visual pigment regeneration PUBMED:14532273.

DISCUSSION

This family represents a retinal pigment epithelial membrane receptor which is abundantly expressed in retinal pigment epithelium, and binds plasma retinal binding protein. The family also includes the sequence related neoxanthin cleavage enzyme in plants and lignostilbene-alpha, beta-dioxygenase in bacteria. (Nicoletti *et al*., 1995). Beta-carotene-15,15'-monooxygenase (BCDO1;) from animals, which cleaves beta-carotene symmetrically at the central double bond to yield two molecules of retinal. Beta-carotene-9',10'-dioxygenase (BCDO2) from animals, which cleaves beta-carotene asymmetrically to apo-10'-beta-carotenal and beta-ionone, the latter being converted to retinoic acid. Lycopene is also oxidatively cleaved. 9-cis-epoxycarotenoid dioxygenase from plants, which cleaves 9-cis xanthophylls to xanthoxin, a precursor of the hormone abscisic acid. Apocarotenoid-15,15'-oxygenase from bacteria and cyanobacteria, which converts beta-apocarotenals rather than beta-carotene into retinal. This protein has a seven-bladed beta-propeller structure with four hisitidines that hold the iron active centre. Retinal pigment RPE65 from animals, which in its soluble form binds all-trans retinol, and in its membrane-bound form binds all-trans retinyl esters. RPE65 is important for the production of 11-cis retinal during visual pigment regeneration.

The predicted structures can be helpful in structural biology for further investigations on allocation of amino acid residues in each fold, prediction of active sites, molecular mechanism of function and structure based phylogeny. The structures were found to be statistically significant by the structure verification programs. The modeling of monoxygenase and doxygenase enzymes from *Danio rerio* gains importance for the structural genomics/ bioinformatics and even to the Fish Biotechnology research from several angles. The present study provides the indispensable groundwork for future Molecular function of all the Beta carotene families of protein.

CONCLUSION

The predicted 3D structures presented here can serve as a guide for the allocation of amino acid residues involved in each fold, which is important for further investigations on molecular mechanism of functions. Retinal pigment RPE65 from animals, which in its soluble form binds all-trans retinol, and in its membrane-bound form binds all-trans retinyl esters. RPE65 is important for the production of 11-cis retinal during visual pigment regeneration. The results presented in this paper will be helpful for further investigation of Retinal pigments in related fish species.

Conflicts of interest: The authors stated that no conflicts of interest.

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