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### RESEARCH ARTICLE

#### IN SILICO ANALYSIS OF DELTA 6 DESATURASE - A KEY ENZYME FOR OMEGA -3/6- FATTY ACID PRODUCTION

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#### Abstract

Delta 6 desaturase is a key enzyme involved in the production of omega 3/6 fatty acids and it is the rate-limiting step. The study aims to characterize the delta 6 desaturase enzyme and to find the binding affinity of various ligand with the protein by docking. It is found that delta 6 desaturase enzyme sequence is very unique and has less similarity with the other desaturase protein. The structural analysis was performed by Ramachandran plot and SCOPE structure prediction. Modeller is used to determine the DOPE score of the selected enzyme. The lowest DOPE score protein is chosen to determine the binding affinity of ligand molecules. Three different ligands were selected and its interaction was determined by the PyRX – Autodock Vina. These studies will give a better idea of the interaction of various molecules, which help to deduce its function by further experimentation.

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#### Introduction:-

Omega 3/6 fatty acids is a polyunsaturated fatty acid mainly required for human nutrition purpose. The deficiency may lead to chronic disorders. The major functions include maintenance of cholesterol, reduction of weight, brain development, anti-inflammatory, etc. Delta-6- desaturase enzyme is a rate-limiting enzyme in the pathway. It is helpful to introduce the double bonds in the fatty acid chain. Different desaturase enzymes are reported in various species and humans also. But each sequence is unique [1]. The sequence similarity is found to be very less. It is found in various organelles.

Fish is the major source of the omega 3/6 fatty acid production [2,3]. Recently, Microalgae is found to be one of the sources for the production of omega 3 fatty acids [4]. So far only one protein was reported in the database. Much research is required for the prediction of proteins involved in the production of these compounds. The present study aims to identify and characterize the delta - 6 - desaturase enzyme. After determining the structure, it is proposed to establish the binding affinity of small molecules with the protein by docking.

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## Methodology:-

### Selection and characterization of enzyme :

The main enzyme responsible for the EPA synthesis is omega -3/6- fatty acid desaturase. This enzyme is selected for the current study. The structure analysis and validation were performed using SCOPe, and SWISS-MODEL analysis. Discrete optimized Protein Structure (DOPE) was calculated from the query sequence along with the similar sequence obtained from NCBI.

### Docking studies:

Docking studies were performed using PyRx software. The ligands are selected for the auto docking with the least DOPE structure. The ligands are ATP (adenosine triphosphate), molecular GOL, NAP, and Gly (glycine). The binding affinity of the docking was predicted and concluded.

## Results and Discussion:-

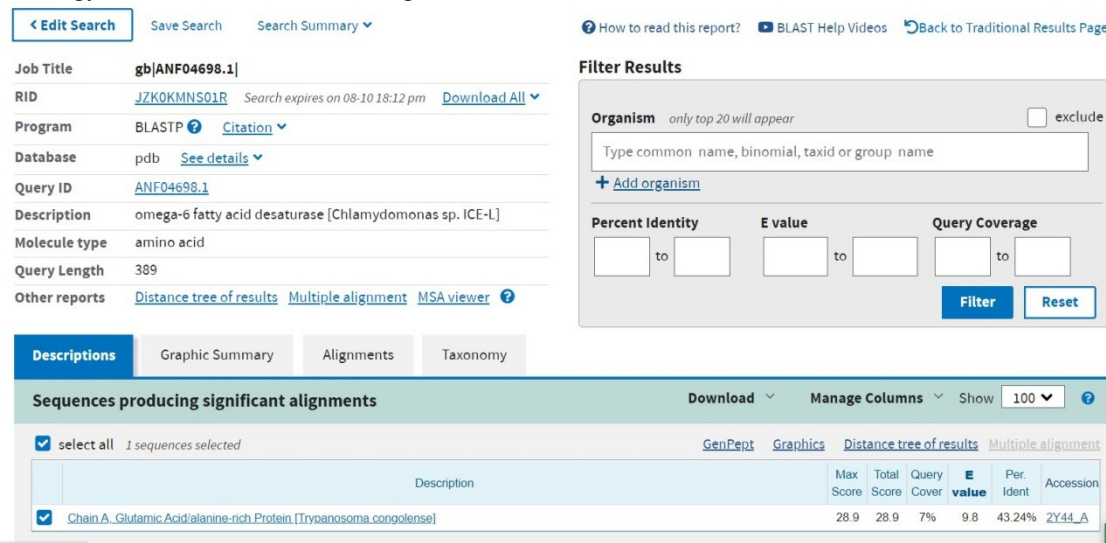
### Selection and Characterization of enzyme.

The omega 3/6 fatty acid desaturase used for the study was given in Table.1. The accession number, FASTA sequence, description was detailed in Table.1. The FASTA sequence was used to find the similar sequence using BLASTP. Only one protein sequence was obtained against the BlastP search (Figure.1). Similar fatty acid desaturase of other organisms also shows less or nil similarity against the PDB database. This indicates the more research is required for the structure prediction and deriving the molecular interaction of this enzyme. The similar other protein was obtained from the PDB database for predicting the DOPE analysis. In addition to 24yy, 6j2y and 4b0n was selected. These are other desaturase enzymes from other organisms. Scope analysis is performed to the structural classification of proteins (Figure.2). The Ramachandran plot statistics were performed using the SWISS-MODEL, and the results are depicted in Figure. 3. It is about 93.5%, which resembles the stability of the structure of the protein.

Modeller 9.24 is used for the DOPE analysis. The sequence alignment is predicted by the Weighted pair-average clustering based on the distance matrix (Figure.4). It shows the 6j2y has some similarity with 24yy than 4b0n. The DOPE score is also given in Figure.5. The least DOPE score model is taken for the docking studies.

### Docking studies:

The ligands were docked with the lowest DOPE score PDB structure. The lowest DOPE score and structure are - 27204 and qseq1.B9990001. Autodock Vina embedded in PyRx is used for the study. Initially, energy minimization is done for all the ligand molecules. ATP is found to lowest binding affinity. Table. 2 represents the binding affinity with the energy minimization value of the ligand.




**Fig. 1:-** Sequence similarity results obtained from NCBI against the query sequence. The percentage identity was only 43.24%.

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### Lineage for d6j2ya1 (6j2y A:1-180)

1. Root: SCOPe 2.07
2. Class b: All beta proteins [48724] (178 folds)
3. Fold b.88: Mss4-like [51315] (2 superfamilies)  
complex fold made of several coiled beta-sheets
4. Superfamily b.88.1: Mss4-like [51316] (5 families) S  
duplication: tandem repeat of two similar structural motifs
5. Family b.88.1.0: automated matches [267622] (1 protein)  
not a true family
6. Protein automated matches [267669] (3 species)  
not a true protein
7. Species Nannochloropsis oceanica [Taxid:145522] [366000] (1 PDB entry)
8. Domain d6j2ya1: 6j2y A:1-180 [366001]  
Other proteins in same PDB: d6j2ya2  
automated match to d1h6qa\_



### Details for d6j2ya1

PDB Entry: 6j2y (more details)

PDB Description: solution structure of translationally controlled tumor protein from photosynthetic microalga nannochloropsis oceanica

PDB Compounds: (A:) NoTCTP

SCOPe Domain Sequences for d6j2ya1:

Sequence; same for both SEQRES and ATOM records: (download)



**Figure.2:-** Structural lineage of the query sequence predicted in SCOPe. Only one PDB entry (6j2y) for Nannochloropsis only reported.



**Figure 3a:-** Ramachandran plot shows the residues present in the most favored region.



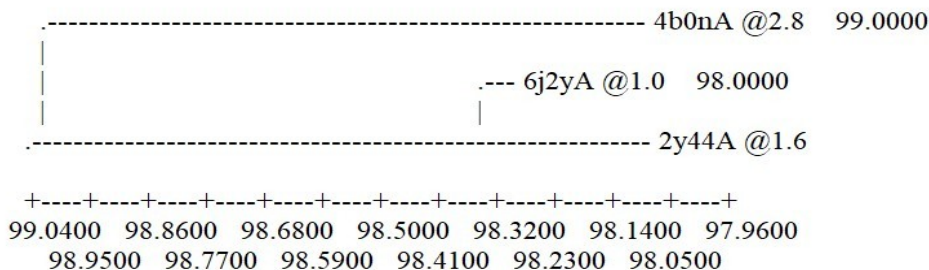
Figure 3b:- Ramachandran plot shows the residues present in the most favored region.

**Sequence identity comparison (ID\_TABLE):**

Diagonal ... number of residues;  
 Upper triangle ... number of identical residues;  
 Lower triangle ... % sequence identity, id/min(length).

4b0nA @26j2yA @12y44A @1			
4b0nA @2	379	2	1
6j2yA @1	1	188	3
2y44A @1	1	2	183

Weighted pair-group average clustering based on a distance matrix:



Total CPU time [seconds] : 0.42

Figure 4:-Sequence similarity obtained from the MODELLER. 6j2y is found to be similar to 2y44.

>> Summary of successfully produced models:

Filename	molpdf	DOPE score	GA341 score
qseq1.B99990001.pdb	3293.73926	-27204.77344	0.01438
qseq1.B99990002.pdb	2772.48730	-28473.67188	0.01646
qseq1.B99990003.pdb	2948.49487	-28575.47461	0.00408
qseq1.B99990004.pdb	2731.12451	-29014.21484	0.01124
qseq1.B99990005.pdb	3101.30591	-28247.81055	0.02273
Total CPU time [seconds]		:	121.66

**Figure 5:-** DOPE scores obtained from the MODELLER. The first one is found to be the least score which is used for docking studies.

**Table 1:-** Details of the omega-6 fatty acid desaturase enzyme, its accession number, aa length and sequence.

Source	Fasta Sequence
Chlamydomonas sp. ICE-L	ANF04698.1 omega-6 fatty acid desaturase [Chlamydomonas sp. ICE-L]
ACCESSION ANF04698	>ANF04698.1 omega-6 fatty acid desaturase [Chlamydomonas sp. ICE-L]
Sequence Length 389 aa	Madrlanmdetqrlvldewgfttvgellpdgadpiilassmsddvrdidggtafralaplalmvsayawmwfmhs IipvwqlvicwiaigtgytglflahdaargaltpsaprlqEflgslimapslysfqawrlrfishiiipnvlglddsawqp VtvealagmgsrarqlarliattprllgslghclhsfgGfdlkeytpdsradilsswavptvfaaaavsvlvvcggis AlsvvwlmpllvfhvwlstltivqhtaphipfkmagldyDlqqaavsgtvtatprwleyilndanyhlpqhdptvp Cyhareateqlrerlpgymteaplsklaniitcwqvydeesntymtfdcaiklieaesaesaesapaqaa

**Table.2:-** Binding affinity and energy minimization values of the different ligand molecules against the protein.

Ligand	Binding Affinity
qseq1.B99990001_ATP_uff E=1652.16	-6.1
qseq1.B99990001_NAP_uff E=2004.40	-5.9
qseq1.B99990001_750_uff E=27.51	-3.9

### Conclusion:-

Omega 3/6 fatty acids is playing a major role in human health. The delta – 6 - desaturase is the rate-limiting enzyme for the production of omega 3/6 fatty acids. The enzyme is found to interact with many molecules in the human body and other organisms. Homology modeling and Ramachandran plot was done to validate the structure of the protein. The docking studies helped to understand the interaction of the protein with different ligands [5]. The complexity of interaction depends upon the organisms and their environmental conditions like stress etc [6]. But this study will form the basis to experimentally reveal the interaction of various ligand with the particular protein. Hence further exploration of interaction will be helpful to overexpress the production of Omega 3/6 fatty acids.

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