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.

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%](image-url)
Figure 2: Structural lineage of the query sequence predicted in SCOPe. Only one PDB entry (6i2y) 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):

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

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

- 4b0nA @2.8 99.0000
- -6j2yA @1.0 98.0000
- -2y44A @1.6

99.0400 98.8600 98.6800 98.5000 98.3200 98.1400 97.9600
98.9500 98.7700 98.5900 98.4100 98.2300 98.0500

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 | -2720.477344 | 0.01438     |
| qseq1.B99990002.pdb       | 2772.48730 | -2847.367188 | 0.01646     |
| qseq1.B99990003.pdb       | 2948.94987 | -2857.47461 | 0.00408     |
| qseq1.B99990004.pdb       | 2731.12451 | -2901.421484 | 0.01124     |
| qseq1.B99990005.pdb       | 3101.30591 | -2824.781055 | 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                                                                      |
| Sequence Length 389 aa        | >ANF04698.1 omega-6 fatty acid desaturase [Chlamydomonas sp. ICE-L]            |
|                               | Madrlanmdetrlvrladwefgftvgepdlpgdpilassmsdvdriddgtfaffralaplamvwsvayawmvfms |
|                               | ipvwqvlwviiagtygtgflahdaargalpsaprkrqeflglslmpsyseqawrlrfishifpnlvglvdsawq |
|                               | vtlsalgamsrarqarlattprllgsllghchhsfgfddkeytqcsrdilswawptvliaaavsilvcggsis |
|                               | Avlswvmpllvlhvwslvkqvqthaipkfiamgdlydjgqasvgtvtaliprwleyidndanyhtpphpqdlpvp |
|                               | Cyhareateqiqrelgpinrneapsilklilanitecqwvydeenstynmtd specifically 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.

References:

1. G.S. Suresha and I.M. Santha, “Molecular cloning and in silico analysis of novel oleate desat urase gene homologues from Brassica juncea through sub-genomic library approach”, Plant Omics., Vol. 6, no. 1, (2013), pp. 55-64.
2. S. Roy, H.J. Chakraborty, V. Kumar, B.K. Behera, R.S. Rana and G. Babu, “In silico Structural studies and molecular docking analysis of Delta 6-desaturase in HUFA biosynthetic pathway”, Animal biotechnology., Vol. 29, no. 3, (2018), pp. 161-173.
3. X. Zheng, M.J. Leaver and D.R. Tocher, “Long-chain polyunsaturated fatty acid synthesis in fish: Comparative analysis of Atlantic salmon (Salmo salar L.) and Atlantic cod (Gadus morhua L.) Δ6 fatty acyl desaturase gene promoters”, Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology., Vol. 154, No. 3, (2009), pp. 255-263.
4. X. Wang, X, Y.H. Liu, W. Wei, X. Zhou, W. Yuan, S. Balamurugan, T.B. Hao, W.D Yang, J.S. Liu and H.Y. Li, “Enrichment of long-chain polyunsaturated fatty acids by coordinated expression of multiple metabolic nodes in the oleaginous microalga Phaeodactylumtricornutum”, *Journal of agricultural and food chemistry*, vol. 65, no. 35, (2017), pp. 7713-7720.

5. M. Kim, B.G. Park, E.J. Kim, J. Kim and B.G. Kim, “In silico identification of metabolic engineering strategies for improved lipid production in Yarrowialipolytica by genome-scale metabolic modeling”, *Biotechnology for biofuels*, Vol.12, no. 1, (2019), pp. 187-200.

6. Barozai, M. Y. K., & Wahid, H. A. (2012). Insilico identification and characterization of cumulative abiotic stress responding genes in Potato (Solanum tuberosum L.). *Pak. J. Bot*, 44, 57-69.