

Computational characterization and structural modulation of Glycerol-3-Phosphate Acyl Transferase relevant to medicinal significance

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Abstract:

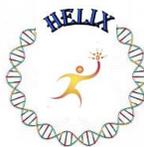
Glycerol 3 phosphate acyltransferase (GPAT, EC: 2.3.1.15) is recognized as a key player of cellular acyltransferase metabolism. It catalyzes the chain initiation step leading to triglyceride biosynthesis. The protein sequence of GPAT was retrieved from GenBank. However, a substantial and well defined structure is not available for this sequence. The 3D structure of GPAT was modeled using a template structure (PDB ID: 1K30) obtained from Protein databank (PDB) identified by searching with position specific iterative BLAST (PSI-BLAST). Modeling was performed using Modeller 9v9 and protein model is hence generated. The GPAT protein model was subsequently docked with specific ligands (Glycerol 3 phosphate, Oleyol-CoA) using AutoDock. The binding of inhibitors to the protein model of GPAT has been discussed taking into consideration the enzyme protein-ligand interaction studies. Besides, the molecular dynamics study of GPAT was carried out using prime module of Schrodinger suit which reveals the stability with the interacting ligands. The results obtained from the present bioinformatics based study provides new insights for lifting up the triglyceride biosynthesis in an oleaginous fungus concomitant with incorporation of unsaturated fatty acids probably be significant from medicinal point of view.

Keywords: *Glycerol 3 Phosphate Acyltransferase (GPAT), Oleaginous fungus, Modeller, Triglyceride, AutoDock, Gromacs.*

Introduction:

Glycerol-3-phosphate acyltransferase (GPAT) catalyzes the transfer of an acyl group from an acyl donor to the sn-1 position of glycerol-3-phosphate. The plant cells contain three types of GPAT, which are located in the chloroplasts, mitochondria and

cytoplasm, respectively ^[1]. Sn-Glycerol-3-phosphate acyltransferase (GPAT) catalyzes the acylation at sn-1 position of glycerol-3-phosphate to produce lysophosphatidic acid (LPA). LPA is an important intermediate for the formation of different types of acyl-lipids, such as extracellular lipid polyesters, storage and membrane lipids ^[2]. Although, The Production of lysophosphatidic acid (LPA) is the first step in the de novo pathway for glycerol biosynthesis, which is mainly catalyzed by the glycerol-3-phosphate acyltransferase (GPATs; EC2.3.1.15). DHAPAT (EC2.3.1.42) also contributes in a minor way, using dihydroxyacetone phosphate as substrate. Final products and intermediate of the glycerolipid synthesis pathway are the main structural components of cellular membranes ^[3] (1 date). LPA is a mitogen that mediates multiple cellular processes including cell proliferation ^[4]. Although, GPAT is involved in triacylglycerol (TAG) and phospholipid synthesis, catalyzing the first committed step. In order to further investigate the in vivo importance of the dominating mitochondrial variant, GPAT1, Female GPAT1 mice had reduced body weight-gain and adiposity when fed chow diet compared with littermate wild-type controls. Furthermore, GPAT1 females on chow diet showed decreased liver TAG content, plasma cholesterol and TAG levels and increased ex vivo liver fatty acid oxidation and plasma ketone bodies. A correlation between reduced mitochondrial GPAT1 activity and decreased proliferation in splenic T-lymphocytes from aged rates. T-lymphocyte proliferation is inhibited and activation induced apoptosis is increased ^[5, 6]. Glycerol-3-phosphate acyltransferase is a rate-limiting enzyme in mammalian triacylglycerol biosynthesis. GPAT is a target for the treatment of metabolic disorders associated with high lipid accumulation. Although the molecular basis for



GPAT1 activation has been investigated extensively.^[7] However Mammals express four isoforms of GPAT. The mitochondrial isoform GPAT1 may have been the acyltransferase that appeared first in evolution. The hepatopancreas of the crustacean *Macrobrachium Borelli* has a high capacity for triacylglycerol (TAG) biosynthesis and storage^[8]. GPAT catalyzes the initial step in the synthesis of all glycerolipids. It is the committed and rate-limiting step and is redundant in *Saccharomyces cerevisiae*, mammals, and plants^[9].

Materials and Methods:

Sequence retrieval

The sequence of the enzyme protein, namely, Glycerol 3 phosphate acyltransferase was retrieved from Gene Bank and template structure was selected using PSIBLAST search with PDB. A suitable template of (PDB_ID:1K30). The selected templates of all the three proteins have more than 67 % sequence similarity.

Modeling of Proteins and ligands

The template of all the proteins was filtered out followed by a proper alignment. After a precise observation for the potential alignment errors, the automated comparative protein modeling program Modeller 9v9. The ligands were designed with the help of canvas module of Schrodinger software, and the energy minimization was carried out using discovery studio 2.5. Ramachandran plot analyses were accomplished with aid of RAMPAGE server.

Docking

The enzyme protein, namely glycerol 3 phosphates, was docked sequentially with ligand using docking server. Besides for revalidation of the results pertaining to docking certain other putative tools namely Q-Site finder was employed for structural Analysis.

Molecular dynamics and Docking

The molecular dynamic study was carried out using GROMACS 4.5 at 20 ps, using CHARMM27 force field in water box. The enzyme protein, namely GPAT, was docked sequentially with ligand using AUTODOCK4.2. Besides for revalidation of the results pertaining to docking certain other putative

tools namely, ARGUSLAB, CHEMSKETCH tool were also used.

Results and Discussion:

The sequence analysis of GPAT is done for its modeling and docking. The docking of GPAT, is performed with G3P and OleoylCoA and the results were analyzed accordingly. The sequence of GPAT was retrieved in FASTA format directly from the net. The FASTA sequence of GPAT enzyme protein is shown below. The template of all the protein was filtered out followed by a proper alignment. After a precise observation for the potential alignment errors, the automated comparative modeling of protein was carried out using a program **Modeller 9v9**.

MODELLER is used for homology or comparative modeling of protein three-dimensional structures. The user provides an alignment of a sequence to be modeled with known related structures and MODELLER automatically calculates a model containing all non-hydrogen atoms.

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MTDSFAHCASHINYRHKMKTMFIFSTPCCSPST
AFFSPFRASNSKPLRSTLSLRSSISSSITSTSHCS
LAFNIVKHKEKNVVSANMTSSVSSRTFLNAQN
EQDVLGIGKEVEAGTLPASIAAGMEEVYLN
KSAVIKSGDPKANEIVLSNMTALLDRIFLDVKE
PFVFEAHHKAKREPFDYMFQGNIRPLVD
TSYVGNMPLFIQMEEQLKQGHNIILMSNHQSEA
DPAIIALLLEMRLPHIAENLIYVAGDRVITVPLC
KPFSGRNLCVYSKKHMLDNPELVDMKRKAN
TRSRKEMAMLLRSGSQIWIAPSGGRDRPVANS
GEWAPAPFDSSSVDNMRRLLVDHSGPPGHIYPL
AILCHDIMPPPLKVEKEIGEKRISYHG TGISTAP
EISFSNTTAACENPEKAKDAYTKALYDSVTEQY
DVLKSAIHGKKGLQASTPVVLSLQPWK
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Using Modeller9v7, the following model of enzyme protein GPAT is generated as shown in Figure 1 by the given sequences. In this process, 20 models were generated and the best model was received on the basis of energy. The protein having the least energy was chosen for carrying out the further studies.

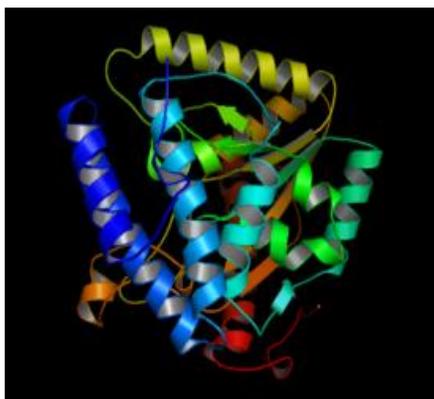
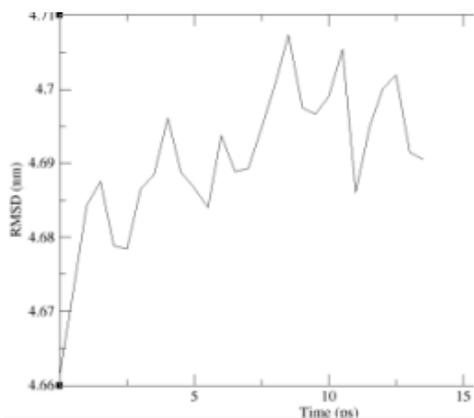


Figure1: Model design of GPAT by using the Modeller 9v9

3.1 Molecular Dynamics analysis of protein:

The molecular dynamics simulation study of GPAT was carried out successfully using GROMACS program (Figure 2, 3 & 4). The analysis of MD studies reflect gradual elevation in the RMSD values (Figure 2) with respect to increase in time and reaches to equilibrium. This nature of graph confirms the stability of the enzyme protein molecule. The Ramachandran Plot studies (Figure 5) show that 90% of the amino acids are present in the core region having 14 alpha-helix and 6 beta-pleated sheets (1 anti-parallel and 2 parallel). And, the radius of gyration of GPAT shows the size of the enzyme protein which is calculated as RMSD from the center of gravity on a given axis. In fact, radius of gyration (Figure 4) shows the structural compactness and higher the compactness, higher is the stability. Thus, radius of gyration reflects the structural compactness with respect to alpha helices and beta sheets.

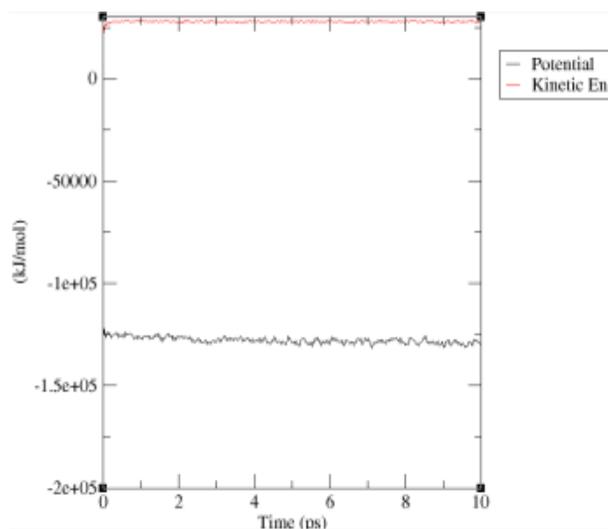
Figure 2: RMSD of the GPAT using gromacs



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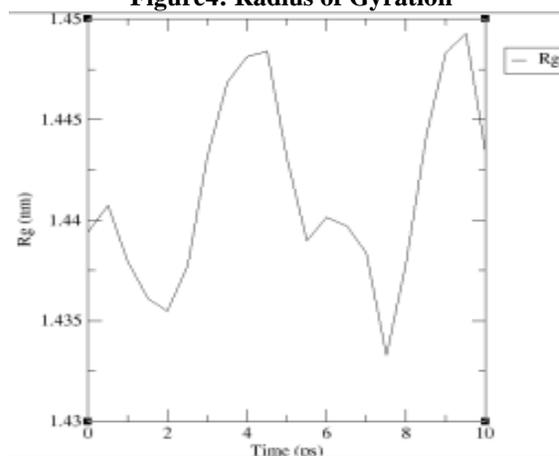
Potential energy and kinetic energy shows the variation as it is representing the structural balance helpful in interaction analysis with ligands.

Figure 3: Potential Energy and Kinetics Energy



Radius of Gyration shows the extent to which the polypeptide chain is folded or unfolded. Upper peaks are showing number of β - sheets while lower peaks are showing α -helix

Figure4: Radius of Gyration



Ramachandran plot (fig 4) showing the relationship between the phi and psi angles of a protein referring to dihedral angles between the N and the C-alpha and

the C-alpha and the C-beta. As an aside, the omega angle between the C-beta and the N tends to be fixed due to pi-pi interactions. Ramachandran plot revealed that the favorable region for 401 amino acids residue is 83 %, and allowed additional region for 71 amino acid is 14.7 %, generously allowed region for 7 amino acid is 1.4 % and disallowed region for 4 amino acid is 0.8%. Therefore the number of non-glycine and non-proline residue are 483 having 100% statistical analyzed participation in docking.

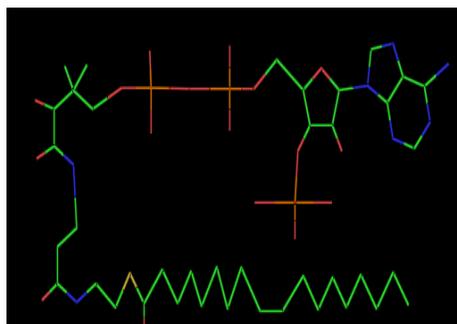


Figure 5: Ramachandran Plot for model assessment

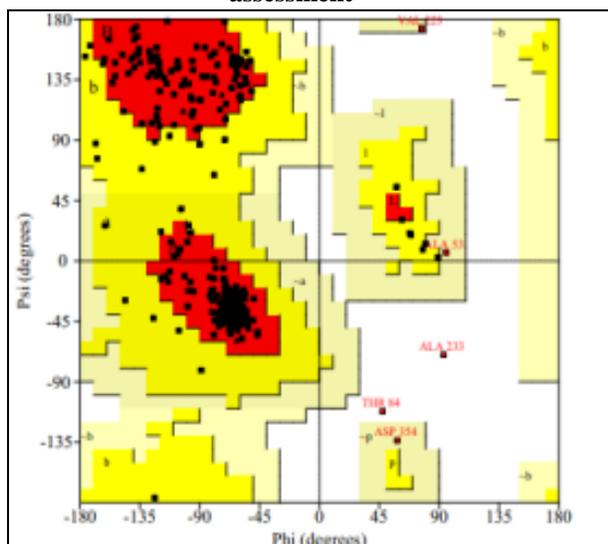
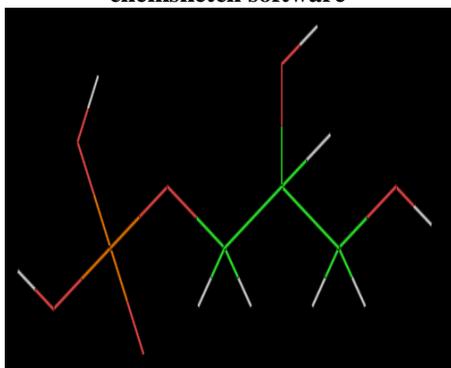


Figure 6: (a) G3P (b) Oleoyl-CoA, designed in chemsketch software



3.2 Docking of GPAT

The docking of ligands G3P and Oleoyl-CoA (Figure 5) with GPAT is successful by estimated binding energy i.e. 4.89 Kcal/mol and 0.39 Kcal/mol (Figure 6), and its electrostatic energy is in negative shows the good binding of ligand as they are polar and non-polar in nature, and overall interaction surface shows the interaction of ligand with protein in positive approach, concluded the successful docking of both the ligands with GPAT protein

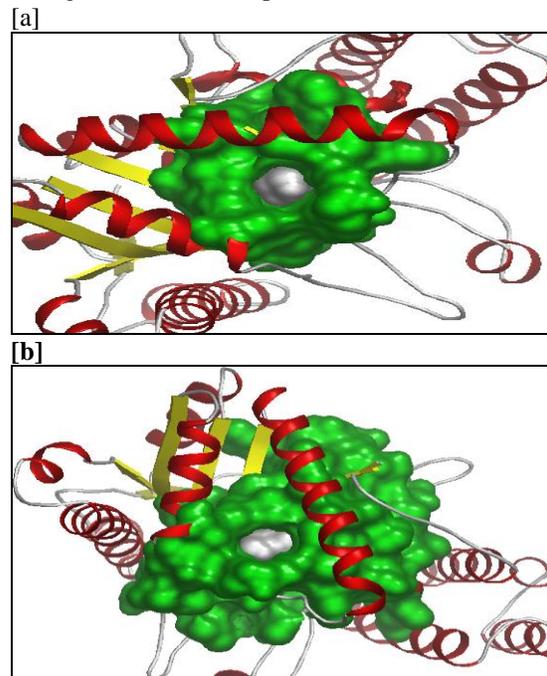


Figure 7: (a) Docking of G3P with GPAT, the gray ligand is grooved inside green protein molecule (b) Docking of Oleoyl-CoA to GPAT, the interaction sites are grey inside the green surface of GPAT.

Conclusion

The present report reflects molecular dynamics simulation and validation of the GPAT protein shows helpful support for further analysis in docking studies, the docking of GPAT protein with G3P and Oleyol-CoA results in interesting docked model with a super coiled secondary structure of the polypeptide chain of the enzyme protein i.e. glycerol-3-phosphate acyltransferase. This unique feature of GPAT projects its activity towards a positive trend. Hypothetically it is mandatory to keep this model stable so that it can be employed for propagating the work in the reagent-based laboratory (*in vivo* as well as *in vitro*) to provide certain platforms for activating GPAT enzyme which may probably be helpful in the elevated biosynthesis of unsaturated fatty acids, ultimately providing a substantial products for pharmaceutically enriched drug molecules.

In summary the polar interactions of G3P (GLU168, GLN230, LYS151) and hydrophobic interaction Oleyol-CoA (VAL139, ALA143, PRO145, LEU180, TYR186, PRO235) are among various biochemically significant amino acids and resulted in the folding mechanism of this pharmaceutically pivotal enzyme leading to specific level of energy of activation for enabling the enzyme to accomplish the same biochemical reaction with rather more potentiality in the due course of time. The data obtained so far may be further useful in monitoring the upgradation of unsaturated fatty acid biosynthesis in the plant tissue culture laboratories. Structure analysis suggests that the size and character of the cavity that is formed from this region determines the specific recognition of acyl chains.

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