In silico Study on the Active Site Conformation and Structural Modulation of Glycerol-3-Phosphate Acyltransferase in Relevance to Medicinal Significance

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Abstract

Glycerol-3-phosphate acyltransferase (GPAT) catalyzes the first step in the synthesis of glycerolipid and glycerophospholipids.Overexpression of GPAT can lead to certain diseases like atherosclerosis and by extension, the risk of heart disease, stroke, obesity and hypertension. The present study was designed to develop specific analogues having high binding affinity for the receptor, probably helping in controlling the overexpression of triacylglycerol (TG) by molecular docking using Vlife MDS. To study the molecular interaction the receptor was docked with GPAT isomers 1IUQ &1K30 after energy minimization. The results obtained after docking indicated formation of a stable complex with strong binding affinity. It was also observed that the amino acid residues involved in this interaction and the predicted residues responsible for binding are GLU124, TYR158, ILE159, ALA160, ASP162, THR163, LEU177 & LEU226 and PRO238, GLY243, TRY245, LYS246, HIS279, ASP280, PRO283, GLU294, ARG296 & ALN299.The molecular interaction between GPAT and its receptor provides new insights into the elucidation of structural domains and development of functional analogues with higher binding affinity and new drug combination therapies for the treatment of obesity.

Keywords: GPAT, Inhibitor designing, Molecular docking, Obesity, TG, Vlife MDS.

1. Introduction

GPAT enzyme (EC 2.3.1.15) catalyzes the first step in TG biosynthesis in most tissues, the conversion of glycerol-3-phosphate and acyl-CoA to 1-acylglycerol-3-phosphate (lysophosphatidate) [1-4]. In 1953 Kennedy and Kornberg first reported on the sn-glycerol- 3 phosphate acyltransferase activity [5-13]. As GPAT exhibits the lowest specific activity of enzymes in the pathway, it has been considered to be rate limiting step of glycerolipid metabolism. GPAT catalyzes the transfer of a fatty acid from an acyl donor to the sn-1 position of glycerol 3 phosphate to yield 1-acylglycerol 3 phosphate (LPA) [14-15]. Subsequently different enzymes function and lead to the formation of triacylglycerol. Triglycerides are the major form of lipids. A triglyceride consists of three molecules of fatty acid combined with a molecule of glycerol. Triglycerides serve as the backbone of many types of lipids [16-17]. In the human body, high levels of triglycerides in the bloodstream have been reported to be associated to atherosclerosis, and, by extension, the risk of heart disease, stroke, obesity and hypertension [18-21]. In eukaryotes, triacylglycerol (TAG) is synthesized through two major pathways, the glycerol phosphate pathway and the monoacylglycerol pathway [22-23]. The glycerol phosphate pathway, first described more than half a century ago, is the major pathway utilized by most

cell types [24]. Acylation of glycerol 3-phosphate occurs through a stepwise addition of fatty acyl groups, each of which is catalyzed by a distinct enzyme (Fig. 1) [9]. By contrast, the monoacylglycerol pathway functions predominantly in small intestine to generate TAG from monoacylglycerol derived from dietary fat [25- 26].
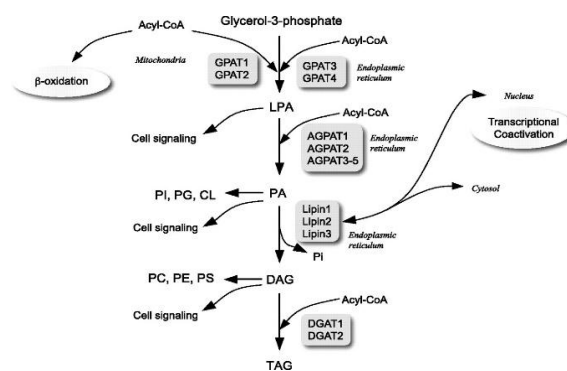
GPAT, glycerol-3-phosphate acyltransferase;
LPA, lysophosphatidic acid;
AGPAT, 1-acylglycerol-3-phosphate acyltransferase;
PI, phosphatidylinositol; PG, phosphatidylglycerol;
CL, cardiolipin; PA, phosphatidic acid; PC, phosphatidylcholine;
PE, phosphatidylethanolamine; PS, phosphatidylserine;
DAG, diacylglycerol; DGAT, diacylglycerol acyltransferase.

Fig 1: Glycerol phosphate pathway for de novo triacylglycerol (TAG) and glycerophospholipid synthesis

GPAT exist in two isoforms viz.1 IUQ and 1K30 (Fig 2) [27-28]. It catalyses the chemical reaction:

Acyl-CoA + sn-glycerol 3-phosphate ⟶ CoA + 1-acyl-sn-glycerol 3-phosphate
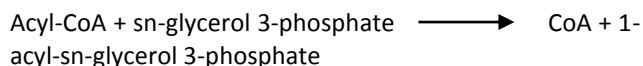
Thus, the two substrates of this enzyme are acyl-CoA and sn-glycerol 3-phosphate, whereas its two products are CoA and 1-acyl-sn-glycerol 3-phosphate[29-30].This enzyme belongs to the family of transferases, specifically those acyltransferase transferring groups other than aminoacyl groups. The systematic name of this enzyme class is acyl-CoA: sn-glycerol-3-phosphate 1-O-acyltransferase. Other names in common use include alpha-glycerophosphate acyltransferase, 3-glycerophosphate acyltransferase, ACP: sn-glycerol-3-phosphate acyltransferase, glycerol 3-phosphate acyltransferase, glycerol phosphate acyltransferase. This enzyme participates in glycerolipid metabolism and glycerophospholipid metabolism. Two isoforms of GPAT are reported (PDB: 1 IUQ, 1 IK30).
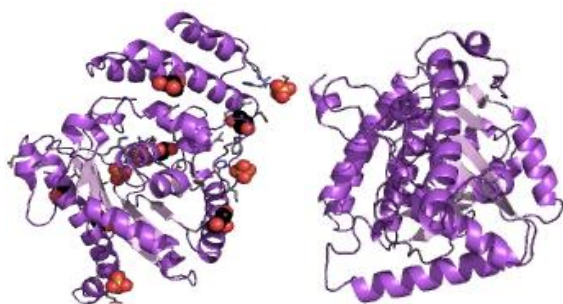

Fig 2: GPAT isomers 1 1UQ & 1 IK30

Overexpression and knock-out studies suggest that GPAT isoforms can play important roles in the development of hepatic steatosis, insulin resistance, and obesity[31-32].From the various previous work several ligands were reported [33-34].Some of them are 15,16-dihydrotanshinones, Cryptotanshinones, Evocarpine, Protein tyrosine phosphate 1B, Quinolones, Tanshinones etc .Through various researches it has been concluded that GPAT can function as a potential target for the treatment of obesity [35-36], and hence the present study was undertaken in view of using molecular docking to design the potent inhibitor for GPAT [37].

2. Material and Methods

2.1 Preparation of the enzyme

GPAT isoforms 1IUQ and 1K30 were retrieved from protein databank (http://www.rcsb.org/) in pdb format, which is further converted to mol2 format by using VlifeMDS 4.3 manual homology modelling module. Firstly, the water molecules were removed from GPAT isomers (1IUQ & 1K30) and hydrogen atoms were added.

Secondly, incomplete and missing amino acid residues were completed by loop builder, finally the optimized geometry was obtained by energy minimization.

2.2 Preparation of ligands

Ligands 3d structure files were downloaded from PubChem databases in .sdf format and converted to mol2 format by using of open babel. Finally the optimized geometry was obtained by minimizing the energy.

2.3 Energy minimization

The energy of both the receptor and the ligand was minimized in order to achieve lowest free energy. The receptor was made free from all other molecules like water and other ligand molecules. Energy minimization was done using VLifeMDS software [38-39]. Various parameters for energy minimization were defined which includes force field (MMFF), charge (Gasteiger Marsili), and maximum number of cycles (10000). Both the receptor and ligand were minimized separately. After energy minimization local geometry check was performed to ensure whether the receptor model was free of any error and suitable for further use.

2.4 Molecular docking

Docking of 26 inhibitors screened from literature against GPAT was done using genetic algorithm (GA) based docking Vlife MDS [40, 43]. The docking method can be used to dock single ligand, which may be treated as flexible with a given receptor. This algorithm offers a strategy for globally searching the docked conformers' space. It follows Darwinian evolution and allows selected population of solution to exist in the next generation. Docking was accomplished at convergence factor of 0.001, 1000 number of generations with dock score as the fitness function criteria and at default values of other docking parameters. Along with the ligands the original substrate for GPAT i.e glycerol 3 phosphate was also docked with the GPAT isomers (1IUQ & 1K30).The ligand and receptor were merged after obtaining the best ligand pose having minimum dock score. The energy of the docked complex was optimized to allow the relaxation of the protein to certain extent, which can account for the conformational changes, happening in the protein structure on binding of the ligand. In addition, the calculated energies were used to estimate the binding energy that helps in quantifying the binding process and to get a better understanding of the molecular recognition and interaction. The free energy of binding was calculated with the formula, and represented in the form of dock score.

$$\Delta G_{binding} = G_{complex} - G_{separated}$$

where $G_{complex}$ and $G_{separated}$ are the free energies of the complex and non-interacting protein and ligand, respectively.

2.5 Structure and function analysis

2.5.1 Active sites of enzyme

BioPredicta provides a utility to find cavities in protein and thereby identify the active site .i.e. the pocket in which ligand is likely to bind. It also provides a utility to find channels surrounding the cavity in protein and thereby identify the active site.

2.5.2 Ramachandran plot

BioPredicta has a utility that displays the Ramachandran plot showing distribution of residues in allowed and disallowed space. A 2D scatter plot was produced showing the backbone conformational angles (phi and psi) with the residue's name.

3. Results and Discussion

3.1 Molecular Docking

The ligands docked with isomers 1IUQ & 1K30 were listed in the Table 1 with the docking score.

**Table 1:** Docking studies of GPAT isomers 1IUQ & 1K30 with ligands

LIGANDS	DOCKING SCORE OF GPAT ISOMERS	
	1 IUQ	1 K30
15,16-dihydrotanshinones	12.0335	-0.9766
Cryptotanshinones	4.4779	-3.8956
Evocarpine	6.8087	-1.7550
Protein tyrosine phosphate 1B	3.6428	-3.3118
Quinolene	1.5416	-3.8956
Tanshinones	6.0447	-3.8956
Tanshinones IIA	4.5619	-3.8956
Taxifolin	3.5521	-3.7010
Terpendoles	7.1852	-3.7010
Tyrophostin-25	0.5587	-3.8956
Sterulic acid	3.7805	-0.9766
Sibutramine	3.8070	-3.1172
Phenethyl alcohol	1.0999	-3.5064
Palmityl CO	1.3948	2.5261
Orlistat	-0.9113	0.1910
n-ethylmaleimide	0.8397	-3.7010
methyl-2-(3-bromo-2-hydroxycyclopentyl)acetate	9.8934	-1.9496
methyl-2-(3-azido-2-hydroxycyclohexyl)acetate	11.6128	-1.5604
methyl-2-(3-azido-2-hydroxycyclohexyl)	8.5573	-1.5604
Malvalic	3.7242	-1.1712
Lsoproterenol	1.3598	-3.1172
Genistein	4.0704	-3.8956
Cerulenin	4.5608	-2.5334
C75	1.1087	-2.3388
Betulin	8.2304	-3.5064
methyl-2-(1R,2R,3R)-2hydroxy-3-(ocyl sulphoamido cyclopenthyl) acetate	11.0019	Maximum affinity towards the substrate-1.9496

It was observed that the ligands cryptotanshinones, tanshinones, quinolone and genistein had the lowest dock score and hence reflecting the highest affinity towards the substrate i.e glycerol 3 phosphate.

3.2 Active site identification

Active site of GPAT isomers, namely, 1IUQ & 1K30 were detected by identifying the amino acids residues interaction with ligands, Docking of GPAT isomers 1IUQ & 1K30 with glycerol-3-phosphate, a natural substrate in Kennedy pathway are shown in Figure 3.

The docking energies were calculated by interaction of ligand with active sites of isomers 1IUQ & 1K30. It was reported that -2.93 and -3.31kcal mol^{-1}binding energy were found for isomers 1 IUQ & 1K30 after optimization. It was found the amino acids residues GLU124, TYR158, ILE159, ALA160, ASP162, THR163, LEU177 & LEU226of 1IUQ were interacting (Fig 3a) with the ligands (listed in Table1). Similarly when the interaction of isomers 1K30 was studied, it was found that the amino acids PRO238, GLY243, TRY245, LYS246, HIS279, ASP280, PRO283, GLU294, ARG296 & ALN299, showed interaction (Fig 3b) with ligands (listed in Table 1).
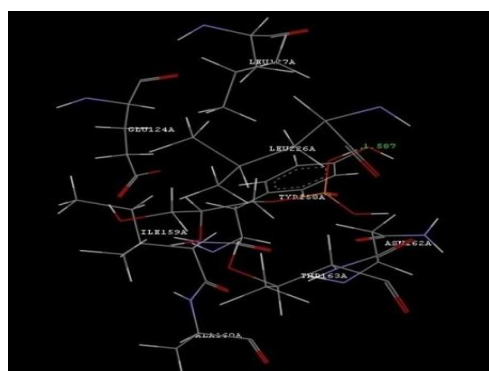
(a)
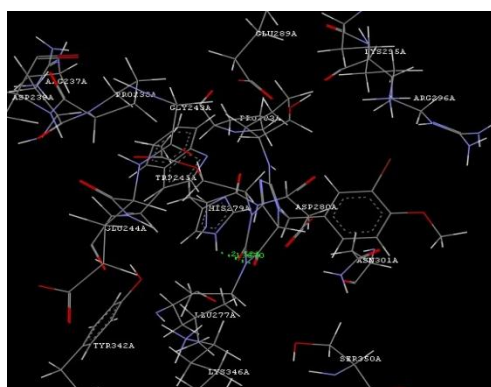
(b)

Fig 3: Interaction of GPAT isomers 1IUQ & 1K30 with glycerol-3-phosphate

3.3 Study the cavity

Cavities generated by Biopredicta module of Vlife MDS represented by blue color (Fig 4). In first part of figure (4a &4b) cavities of isomer 1IUQ were represented, it was found that the presence of two cavities, which populate the residues list with names of residues that are in the vicinity of the selected cavity (cavity1concludes10207 & cavity 2 concludes 2018 points). The second part of figure (4c & 4d) represent isomer 1K30 with two cavities (cavity1concludes 11044 & cavity 2 concludes 3328 points) [41-42].
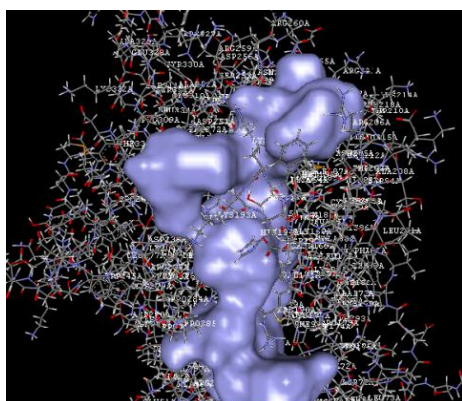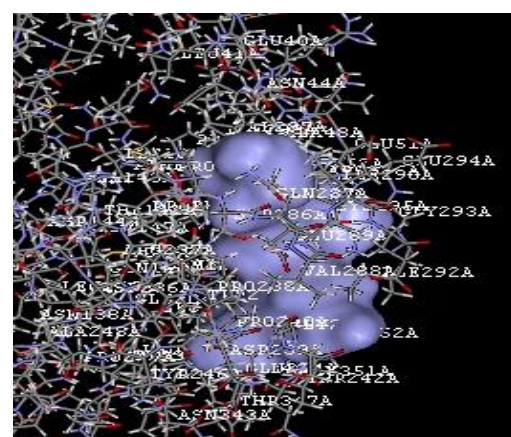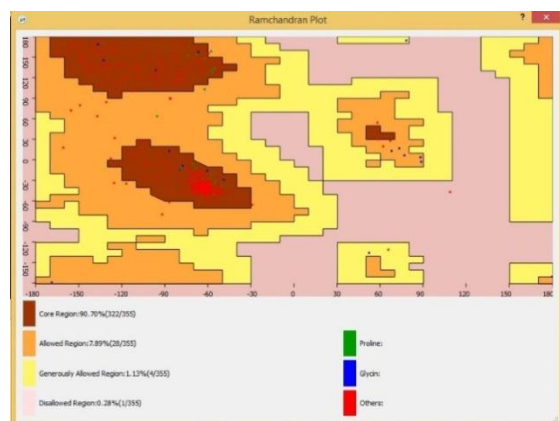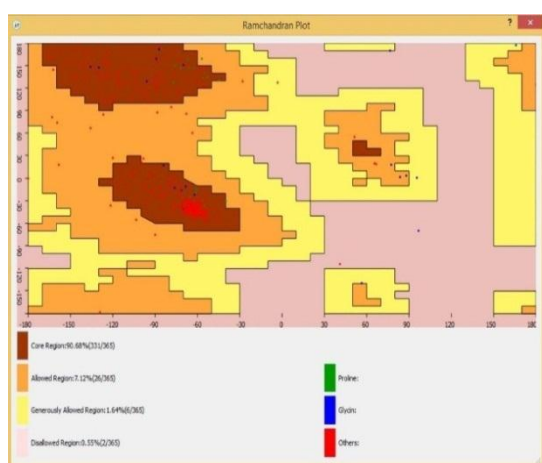

(a)
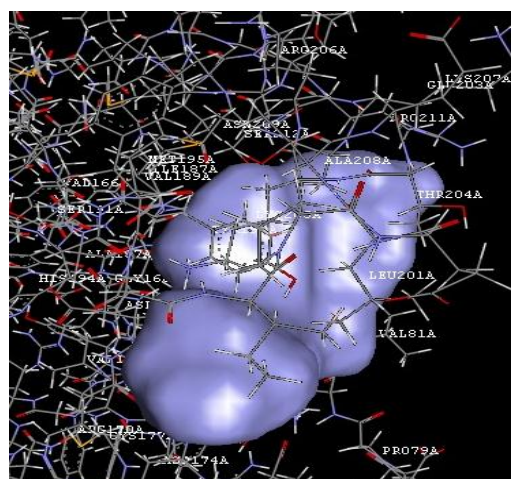
(b)
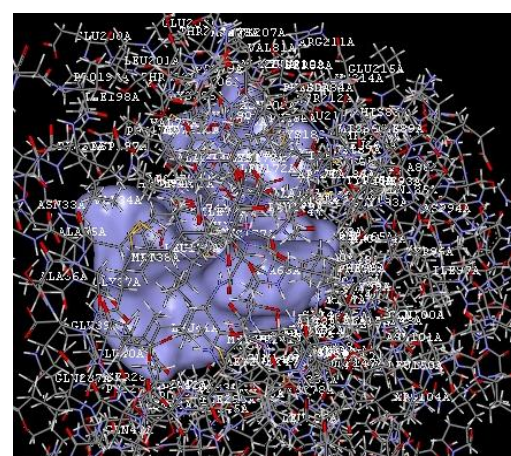
(c)

(d)

Fig 4: Binding cavities of GPAT isomers 1IUQ (4a, 4b) & 1K30 (4c, 4d)

3.4 Ramachandran plot

The Ramachandran plot of isomers 1IUQ & 1K30 were studied using Vlife MDS (Fig 4).

(a)

(b)

Fig 4: Ramachandran plot of isomer 1IUQ (a) & 1K30 (b)

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Ramachandran Plot Analysis for - 1IUQ

Core Count               :322/355 (90.70%)
Allowed Count            :28/355 (7.89%)
Generously Allowed Count :4/355 (1.13%)
Disallowed Count         :1/355 (0.28%)
* Note - All counts are including GLY & PRO residues
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Ramachandran Plot Analysis for - 1K30

Core Count               :331/365 (90.68%)
Allowed Count            :26/365 (7.12%)
Generously Allowed Count :6/365 (1.64%)
Disallowed Count         :2/365 (0.55%)
* Note - All counts are including GLY & PRO residues
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Conclusion

In this molecular interaction study we have docked various ligands with this receptor model after energy minimization. Dock score and the binding energy obtained after optimization of the docked complex indicated formation of a stable complex and strong affinity binding towards the enzyme isomers. It was found that amino acid residues lying in 1IUQ and 1K30 were mainly involved in the interaction and the most important residues predicted to be involved in these interactions are GLU124, TYR158, ILE159, ALA160, ASP162, THR163, LEU177 & LEU226 and PRO238, GLY243, TRY245, LYS246, HIS279, ASP280, PRO283, GLU294, ARG296 & ALN299 respectively. Conclusively, the present study concerning with the molecular interaction between GPAT and its receptor provides new insights into the elucidation of structural domains and development of functional analogues with higher binding affinity and new drug combination therapies for the treatment of obesity.

Acknowledgement

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