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Structural Characterization of Human 15-Lipoxygenase Protein, A Key Player in Human Pancreatic Cancer

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ABSTRACT

Lipoxygenase are a group of enzymes that are involved in fatty acid oxidation in plant, animal and mammalian cells. All lipoxygenases are known to be pharmaceutically important, seen to mediate inflammatory and cancer disorders. In this study, the homology modeling methodology is used to model the human 15-Lox which plays an important pathological role in all types of cancer. Based on NCBI blast search a template protein 1-lox was chosen with whom the target protein sequence identity was seen to be 81%. The target protein modeled using the modeller 9v9 program; followed by refinement through energy minimization processes and validated by saves server. Analysis of the Ramachandran plot shows 99.98% amino acids fall in the allowed region, illustrating the model structure to be accurate. The modelled protein was docked with the seven ligand compound using the schrödinger maestro 9.2 tool and results showed the masoprocol and NDGA legend have good interactions. Both masoprocol and NDGA ligands are conformers and bind with the same amino acid in the binding site of the modeled protein. The docking score for masoprocol was -7.53, and for NDGA -7.12 with the modeled protein. Both the docked protein ligand complexes were refined and stabilized by molecular dynamic simulation in Desmond's. The simulation results, potential energy and RMSD graph showed the protein-ligand complexes are stabilized at 3nsec.

Keywords: Lipoxygenase, *Molecular docking, Molecular dynamics*

1. INTRODUCTION

Lipoxygenase is a member of LOX family, which is expressed in plants, animals, fungi and in some bacteria. It catalyzes the di-oxygenation of poly-unsaturated fatty acid with the help of lipid substrate and atmospheric oxygen in this-1,4-pentadine structure [1].

A recent study suggests that cancer is characterized by a poor prognosis and lack of response to conventional therapy. Many studies have shown an association of pancreatic cancer development and growth with high dietary fat intake, especially intake of n-6 polyunsaturated fats. A growing body of evidence suggests that specific metabolites of the arachidonic acid act as an important element in signaling pathways necessary for cancer cell transformation, growth and metastasis, via lipoxygenase (LOX) pathways. The proliferation of pancreatic cancer cells is inhibited by LOX inhibitors and stimulated by LOX metabolites. The direct stimulation of pancreatic cancer cell proliferation by the metabolites of 12 -LOX and 15- LOX (12-HETE and 15-HETE, respectively) is mediated by multiple signaling pathways [2].

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Mammalian lipoxygenase possess region-specificity for interaction with substrate, and on this basis have been designated as arachidonate 5, 12 or 15-lipoxygenase (5-LOX, 12-LOX, and 15-LOX)[4-6]. The three distinct enzyme's insert oxygen at carbon 5, 12 or 15 of arachidonic acid, and the primary product is 5S, 12S or 15S-hydroperoxyl satetraenoic acid (5,12 or 15-HPETE), which can be further reduced by glutathione peroxidase to hydroxy forms (5,12,15-HETE)[2-5,15]. Lipoxygenase is involved in the formation of unattached primary, intermediate, which remain enzyme bound. So, lipoxygenase reaction may not be considered as a source of free radical.

The 15-LOX enzyme also produces 13-S-hydroxyoctadecadienoic acid (HODE) from linoleic acid. These lipid products have a variety of functions in human tissues. The product of 12 /15-LOX is 12 (S) -HETE and 15 (S) -HPETE are involved in monocyte binding in the vasculature, by stimulating protein kinase C (PKC) and various cellular adhesion molecules (CAMs). The lipoxins are synthesized from AA by 5-, 12-, and 15-LOX, as well as by COX-2 in the presence of aspirin [7-

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2. MATERIAL AND METHODS

2.1. Molecular Modeling

Homology modeling was used to generate the 3D structure of the proteins. Protein sequences for Lipoxygenase (662 amino acids, UniProtKB accession number (P16050)) were retrieved from UniProt (http://www.uniprot.org). Protein BLAST (BLAST-P) was conducted against the Protein Data Bank (PDB, http://www.rscb.org/pdb/) using query sequences to search for structural templates. The closest structure of Human Lipoxygenase (PDB ID:1LOX) from Rabbit Reticulocyte with 81% sequence identity was selected as the protein template for homology modeling. The suitable model was minimized by Swiss PDB Viewer tool and then verified using the Ramachandran plot to examine the possible conformations of ψ and φ angles of amino acid residues in protein structure.

2.2. Molecular docking

2.2.1. Protein and ligand preparation

The Protein-Ligand docking interaction was performed by using the Schrödinger maestro9.2 suite. Initially, we prepared the protein using the default parameters in Protein preparation wizard. The required chain of the protein structure was retained and others are deleted, along with the explicit and the un-interacted water molecules, unwanted ions, etc. Then the protein structure was optimized and minimized by using the 'Impref minimization" by using 'OPLS 2005' force field.

Further, ligand compounds were retrieved from the different database and imported to the LigPrep wizard for ligand preparation. It generates the possible ionic states of the all the molecules. OPLS2005 was taken as the force field for minimize the structures. Minimized structure was further imported to glide for molecular docking.

2.2.2. Molecular docking by Using Glide

Receptor grid generation is the first step of the docking process in which the program will generate Grid Box around the active site of the protein molecule. Receptor ligand docking is the process of molecular docking in which the protein molecules acts as a receptor and small molecule as a ligand. The XP (Xtra Precession) docking was also performed for those molecules to finding the interactions between protein and ligand molecule, the Receptor ligand docking was done by the analysis of these results and scores, the interactions we can conclude that which ligand can be said as the best possible drug like compound for the receptor.

2.2.3. Molecular Dynamic Simulations

Desmond is the high performance molecular dynamics simulations for bio-molecular systems. It uses novel parallel algorithms and numerical techniques to achieve high performance accuracy on platforms containing a large number of processors, but may also be executed on a single computer. Based on the g_score, the best binding pose were analyzed.

3. RESULTS AND DISCUSSION

Homology modeling develops a three-dimensional model from a protein sequence based on the structures of homologous proteins. High sequence identity (>81%) between target and template sequence ensures the quality of modeled protein.

Sequence alignment with the template gives the information about the sequence conservation and signature motifs.

3.1. Molecular Docking analysis

Ligands which are obtained from the database screening are used to form a ligand library. The results of the Docking studies can be studied by the analysis of the number of hydrogen bonds the legend is formed with the receptor active site residues and the number of hydrophobic interactions also play an important role in the Complex between protein and ligand. The strength of the interaction can also be analyzed by the scoring of each possible interaction. The ligand or compound giving the highest negative score is chosen as a best ligand and further analysis is performed by taking it with the receptor protein.

The active site obtained from the literature is similar to the interactive site observe in the docked complex of protein and ligand molecule. All ligands are bound at defining the active site of the protein. Out of seven ligands only two ligand compound show a good docking score, which has been considered as a lead compound. The Masoprocol compound is taken from DrugBank database (ID: db00179) and another NDGA (Nordihydroguaiaretic acid) ligand from the pharmaceutical industry. Both ligands interact with protein at residue no. 600 (side chain), 174, 662

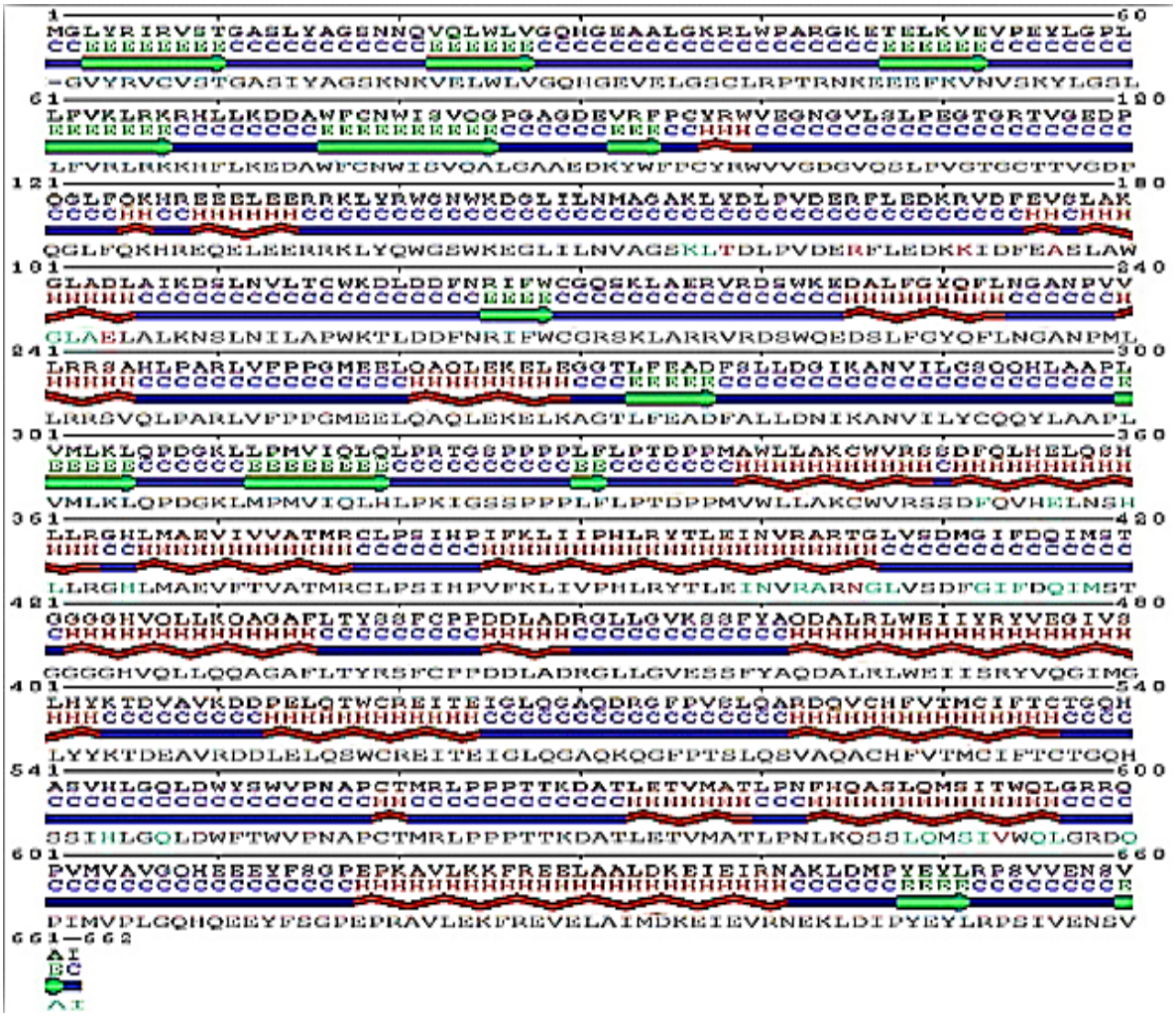


Figure1. In the secondary structure of protein 21 helixes, 13 B-sheet are present in protein structure.

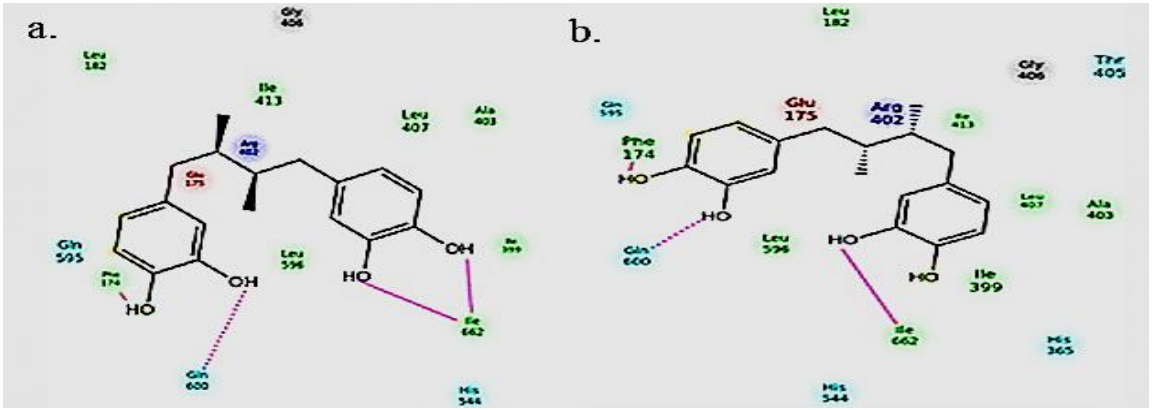
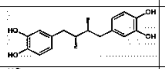
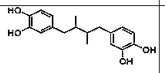
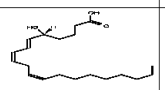
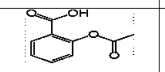
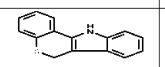
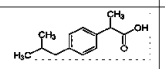
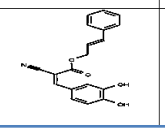


Figure2. Showing interaction of ligand with amino acid of LOX protein.

Table1. Result obtains from docking of protein with all seven ligands.

LIGAND NAME	LIGAND STRUCTURE	GLIDE SCORE	H-BOND ENERGY	GLIDE evdw	Electrostatic ENERGY
Masoprocol		-7.53	-3.33	-2.95	-1.19
NDGA		-7.12	-2.73	-2.84	-1.14
HETrE		-4.56	-1.05	-3.77	-0.37
Aspirin		-3.99	-0.35	-2.78	-0.15
PD_146176		-3.78	0.00	-3.28	-0.01
Ibuprofen		-2.99	-0.35	-2.42	-0.06
CDC		-2.67	-0.96	-3.68	-0.62

(back bone) by forming hydrogen bond and Hydrophobic interaction. Both ligand molecules are bound near by the Fe(II) ion present in an active site of protein, but NDGA is nearer to the metal and masoprocol is far from metal. Both the ligands molecule exhibits an identical structure with same molecular. Both are optical isomer and FDA approved drug, available as anti-neoplastic drug to treat skin growths caused by sun exposure.

3.2. Structural model refinement by molecular dynamic simulation

The Molecular Dynamic analysis concerned the stability of simulated LOX protein with Masoprocol ligand during the simulation by monitoring the RMSD profiles as computed by the atomic displacements from Molecular Dynamic trajectories. It shows that RMSD value rises in first 1Å till 2Å and then remains quite stable up to 2.5Å in the simulation time. Highest peak was observed at 2. 5Å (Fig. 3). Highest peak was observed at -214600kj energy at 0ps and after that it decreases. The energy graph shows the gradual decrease in potential energy of the model from -214600kJ/mol to -215600kJ/mol at 3000ps, which indicates that the model is energetically stable during molecular dynamic simulation. RMSF graph also decreasing gradually from 3.75 to 0.8 and the lower RMSF value obtain at 0.7 and again increase from 0.8 to 3.6.

The stability of simulated LOX protein with NDGA ligand RMSD value rises in first 1Å till 2Å and then remains quite stable up to 2.5Å in the simulation time. The energy graph shows the gradual decrease in potential energy of the model from -214600kJ/mol to -215400 kJ/mol at 3000ps,

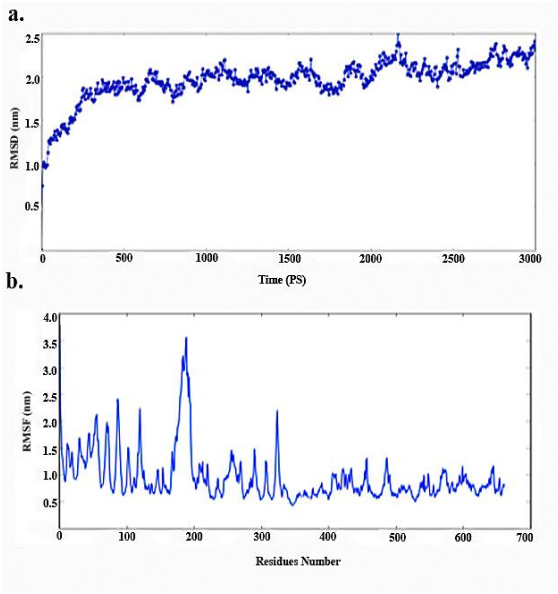


Figure 3. RMSD graph for the protein - Masoprocol complex. b. RMSF graph for protein-Masoprocol complex.

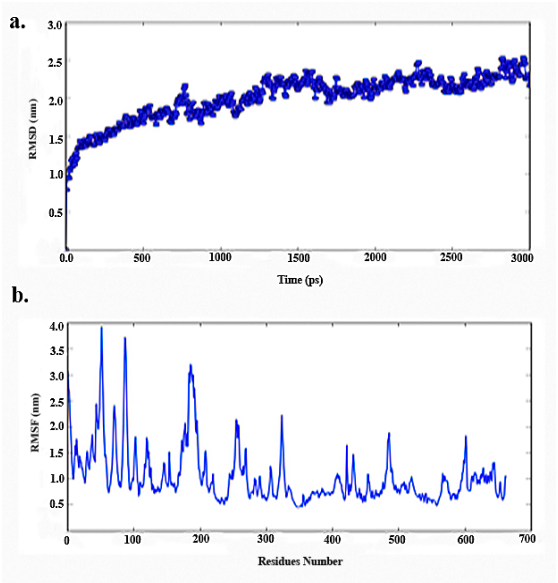


Figure 4. RMSD graph of protein-NDGA complex. b. RMSF graph of protein-NDGA complex.

which indicates that the model is energetically stable during molecular dynamic simulation. RMSF graph also decreasing gradually from 3.10 to 0.9 and lower RMSF value is obtained at 0.7 and again increase from 0.9 to 3.9.

4. CONCLUSION

The existence of a large super family of proteins that carry out the same chemical reaction (oxygenase) on a wide variety of lipoxygenase substrate have common structural element and are combined with unique features to generate the systemic diversity. The process of the inhibitor finding through structure based drug designing (SBDD) of the Human LOX (15-Liopxygenase)

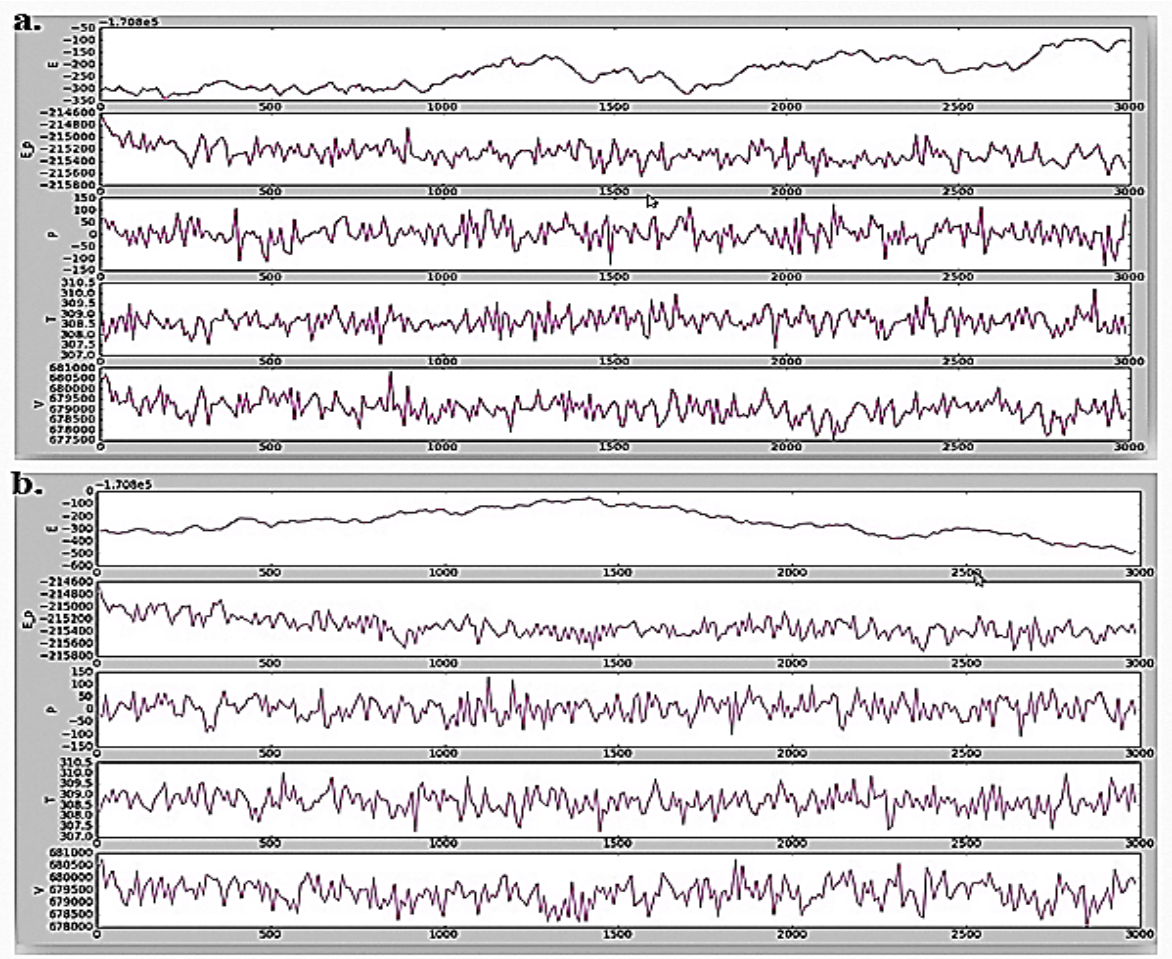


Figure 5. Molecular dynamics analyses for the protein ligand complex refinement. Energetics graph shows the energetically stable conformation constant Temp., Volume and Pressure for the protein - Masoprocol complex. b. Molecular dynamics analyses for the protein ligand complex refinement. Energetic graph shows the energetically stable conformation constant Temp., Volume and Pressure for the protein - NDGA complex.

protein has revealed the possibilities of new inhibitors. Docking studies show that the ligands bind active site, which consists of a non- heme iron and the binding site of the protein structure is a unique one. Only two lead molecules show the better interaction with the protein molecule. The complex formed between them is stronger. This can be analyzed through their docking scores and the intermolecular interactions between the ligand protein complex which is also seen to be highly stable as it has the highest hydrogen bonds and more hydrophobic interactions. The Molecular Dynamic Simulation (MDS) shows that model protein is steady throughout the total time scale of the 3ns, and the potential energy is also decreased. This is the sign of the fixed complex at constant temperature and pressure. This shows that a protein ligand compound is stable. Both lead compounds can be used to inhibit the Lipoxygenase protein and its activity.

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****Bibliographical Sketch***

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