J Comput Aided Mol Des (2008) 22:611–619
DOI 10.1007/s10822-008-9180-0

Homology modeling of 5-lipoxygenase and hints for better inhibitor design

P. Aparoy · R. N. Reddy · Lalitha Guruprasad · M. R. Reddy · P. Reddanna

Received: 24 October 2007 / Accepted: 10 January 2008 / Published online: 30 January 2008

Abstract Lipoxygenases (LOXs) are a group of enzymes involved in the oxygenation of polyunsaturated fatty acids. Among these 5-lipoxygenase (5-LOX) is the key enzyme leading to the formation of pharmacologically important leukotrienes and lipoxins, the mediators of inflammatory and allergic disorders. In view of close functional similarity to mammalian lipoxygenase, potato 5-LOX is used extensively. In this study, the homology modeling technique has been used to construct the structure of potato 5-LOX. The amino acid sequence identity between the target protein and sequence of template protein 1NO3 (soybean LOX-3) searched from NCBI protein BLAST was 63%. Based on the template structure, the protein model was constructed by using the Homology program in InsightII. The protein model was briefly refined by energy minimization steps and validated using Profile-3D, ERRAT and PROCHECK. The results showed that 99.3% of the amino acids were in allowed regions of Ramachandran plot, suggesting that the model is accurate and its stereochemical quality good. Like all LOXs, 5-LOX also has a two-domain structure, the small N-terminal β-barrel domain and a larger catalytic domain containing a single atom of non-heme iron coordinating with His525, His530, His716 and Ile864. Asn720 is present in the fifth coordination position of iron. The sixth coordination position faces the open cavity occupied here by the ligands which are docked. Our model of the enzyme is further validated by examining the interactions of earlier reported inhibitors and by energy minimization studies which were carried out using molecular mechanics calculations. Four ligands, nordihydroguaiaretic acid (NDGA) having IC_{50} of 1.5 μM and analogs of benzyl propargyl ethers having IC_{50} values of 760 μM, 45 μM, and no inhibition respectively were selected for our docking and energy minimization studies. Our results correlated well with the experimental data reported earlier, which proved the quality of the model. This model generated can be further used for the design and development of more potent 5-LOX inhibitors.

Keywords Homology modeling · 5-LOX · Soybean LOX-3 · Molecular docking · Benzyl propargyl ethers

P. Aparoy · P. Reddanna (✉)
School of Life Sciences, University of Hyderabad, Hyderabad 500 046, India
e-mail: prsl@uohyd.ernet.in

R. N. Reddy · M. R. Reddy
Rational Labs (P) Limited, Plot # 177, IDA Mallapur, Hyderabad 500 076, India

L. Guruprasad
School of Chemistry, University of Hyderabad, Hyderabad 500 046, India

Introduction

Lipoxygenases (linoleate: oxygen oxido reductase, EC 1.13.11.12) are a group of closely related non-heme iron containing dioxygenases. These enzymes catalyze the addition of molecular oxygen into polyunsaturated fatty acids (PUFAs) containing *cis*, *cis* 1–4 pentadiene structures to give their hydroperoxy derivatives. Lipoxygenases (LOXs) are essentially ubiquitous among eukaryotic organisms and have been demonstrated to exist in many tissues of numerous fungi, higher plants and animals [1–4]. Higher plants and animals contain multiple LOXs with at least eight identified in soybean, seven in mouse and five in humans [5–7]. High levels of LOX expression is observed

in few plant and animal tissues. They constitute a major portion of the proteins in soybeans, and 15-lipoxygenase (15-LOX) represents one of the main proteins in rabbit reticulocytes during anemia [8]. Soybean and potato are the well-characterized LOXs in plants [9–12].

Polyunsaturated fatty acids containing a series of *cis* double bonds act as suitable substrates for LOXs. LOXs are classified according to their positional specificity of arachidonate oxygenation into 5-, 8-, 9-, 11-, 12- and 15-LOXs [13]. The prominent animal LOXs are 5-LOX, 8- LOX, 12-LOX and 15-LOX, while the plant LOXs are mostly 5-LOX and 15-LOX. The differing chain lengths of the most common substrates of plants (linoleic and α-linoleic acids,18-carbon) and animals (arachidonic acid, 20-carbon) result in a plant 13-LOX or 9-LOX corresponding to a mammalian 15-LOX or 5-LOX. There are limitations in obtaining sufficient quantity of the purified 5-LOX in stable form from mammalian sources, so potato tubers are frequently employed as the source for large amounts of 5-LOX [11] in screening specific inhibitors.

Lipoxygenase proteins have a single polypeptide chain with a molecular mass of 75–80 kDa in animals and 94–104 kDa in plants [14, 15]. All LOXs have a two-domain structure, the small N-terminal β-barrel domain and a larger catalytic domain containing a single atom of non-heme iron. The catalytic iron is ligated in an octahedral arrangement by three conserved histidines, one His/Asn/Ser, and the C-terminal isoleucine [16]. The highest sequence identity between these LOXs is in the portion of the catalytic domain near the iron atom [17]. Though most of the lipoxygenases insert molecular oxygen stereospecifically at 'S', recently 'R' lipoxygenases also have been reported [7, 18].

One of at least four LOX pathways of arachidonic acid metabolism, the 5-LOX pathway is the source of potent pro-inflammatory mediators [14]. LOX metabolites are potent physiological effectors in a variety of cellular responses, associated with normal host defense and inflammation. In particular, leukotrienes (LTs), the mediators of allergy and asthma, are produced through the 5-LOX pathway. The 5-LOX acts preferentially upon unesterified arachidonic acid, inserting molecular oxygen at the fifth carbon and forming the hydroperoxy intermediate, 5-hydroperoxyeicosatetraenoic acid (5-HPETE) [19, 20]. The same enzyme then catalyzes a dehydration reaction, forming the unstable epoxide intermediate, leukotriene A_4 (LTA_4). In intact inflammatory cells, the presence of 5-lipoxygenase activating protein (FLAP) is required to make this enzyme active [21]. LTA_4 can then be further metabolized to LTB_4 by LTA_4 hydrolase or to LTC_4 by conjugation of glutathione at the sixth carbon by the action of LTC_4 synthase [22]. Additional studies established that LTC_4 and its extracellular metabolites LTD_4 and LTE_4 are the constituents of slow-reacting substance of anaphylaxis, but they are now more properly termed as cysteinyl leukotrienes. The cysteinyl leukotrienes have been recognized to mimic many of the clinical manifestations of asthma, including sustained bronchoconstriction, hypersecretion of mucus, and airway edema. LTE_4 gets further metabolized to inactive LTF_4 by the action of γ-glutamyl transpeptidase. Also LTF_4 was shown to be formed directly from LTC_4 by the action of carboxypeptidase [23].

Products of the 5-lipoxygenase pathway are thus important mediators of inflammation. Inhibitors of the 5-LOX pathway, therefore, have a therapeutic potential in a variety of inflammatory and allergic diseases. These efforts have resulted in the release of Zileuton (5-LOX inhibitor) and Montelukast (leukotriene receptor antagonist) into the market for the treatment of asthma [24]. Recent studies have indicated that LOX inhibitors may be superior to leukotriene-receptor antagonists, as they block the action of the full spectrum of 5-LOX products whereas leukotriene receptor antagonists would produce narrower effects. We have reported the inhibition of potato 5-LOX by Vitamin E [25] and benzyl propargyl ethers [26]. Currently an emerging strategy of therapeutic value consists of creating molecules with specific 5-LOX inhibition activity. In a view to help in the design of such compounds, structural insights into 5-LOX active site and its interaction with ligands are essential. As the crystal structure of the protein is yet to be elucidated, modeling studies will be helpful to obtain a consistent representation of the enzyme-inhibitor recognition events at the molecular level and furthermore, provide new insights that can be used to design novel therapeutic agents. The conserved structural pattern of all lipoxygenases mainly at the active site residues formed an advantage for building up a reasonable homology model. The structure can provide hints about functional and evolutionary features of the protein and is also useful in drug design efforts.

To our best knowledge, the three dimensional (3-D) experimental structure of 5-lipoxygenase is not known. To date, structural information for only three lipoxygenases is available from the Protein Data Bank of Brookhaven (PDB, www.rcsb.org/pdb): two isoforms from soybeans, LOX-1 and LOX-3, and one from rabbit reticulocytes, 15-LOX. Homology modeling was used in generation of 3-D model of human 5-LOX. [27–31] In our present study, homology modeling technique was used to built the 3-D model of potato 5-LOX based on the known structure of soybean lipoxygenase- 3 complexed with 4-nitrocatechol (PDB ID: 1NO3) [32]. The 3-D model of potato 5-LOX is used to search the active site and carry out the binding studies by flexible docking and energy minimization methods with the benzyl propargyl ether ligands (inhibitors). Our results may be helpful for further experimental studies on 5-LOX and discovery of more selective inhibitors of 5-LOX.

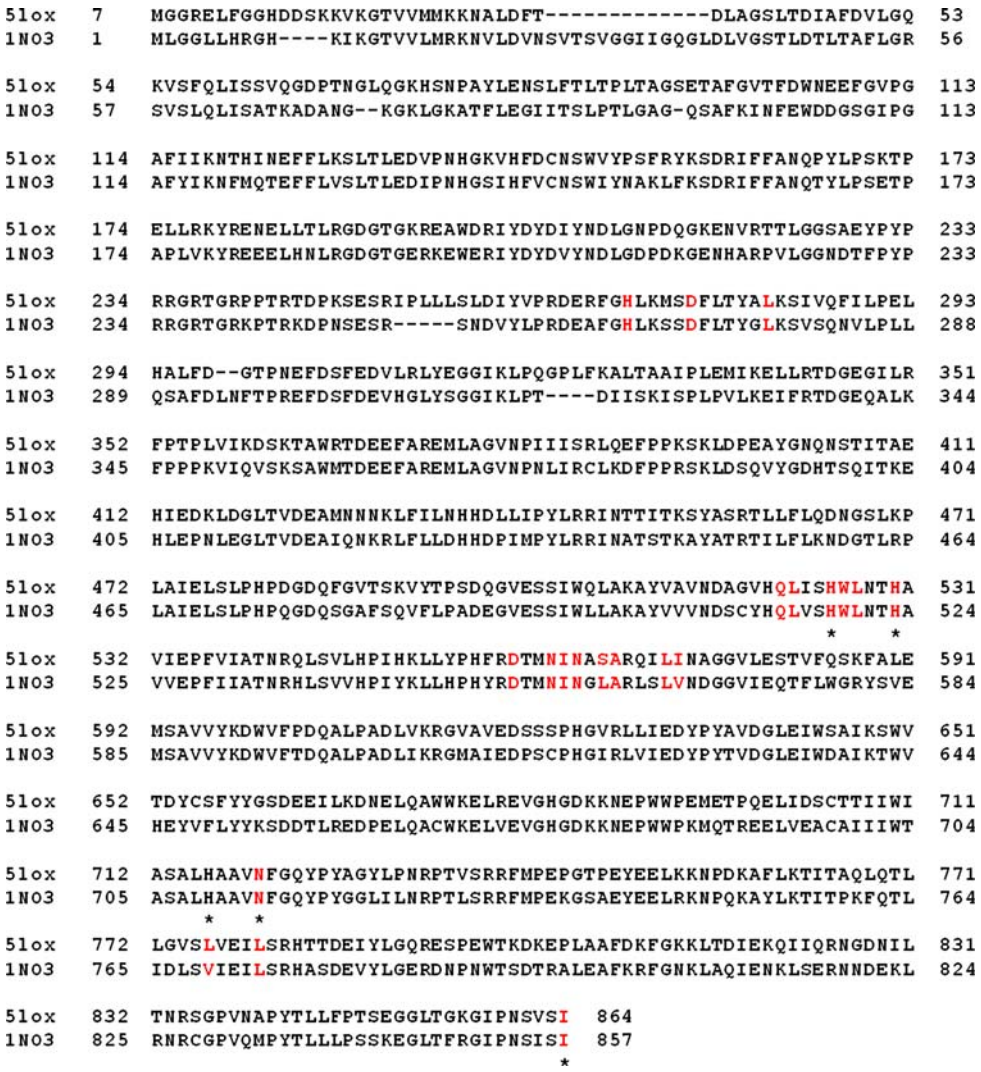


Fig. 1 Alignment of soybean LOX-3 (1NO3) and potato 5-LOX sequences showing 61% identities and 75% positives. Essential sites for iron binding are denoted by asterik (*) and amino acids at the binding site are shown in red color

Materials and methods

Construction and refinement of the structure model

All simulations were performed on the SGI workstation by using InsightII software from Accelrys, USA [33]. The amino acid sequence of potato 5-LOX (Accession Id: AAD04258) is obtained from the databank in the National Center for Biotechnology Information, NCBI (www.ncbi.nlm.nih.gov). The BLAST program against Protein Data Bank (PDB) available at NCBI was used to select a template structure for homology modeling of potato 5-LOX.

The crystal structure of soybean lipoxygenase-3 (PDB code: 1NO3) was obtained from PDB database [34] and it shares 63% sequence identity with 5-LOX as shown in Fig. 1. The first requirement in the construction of 5-LOX model is the sequence alignment among these templates. The sequence alignment is based on identifying structurally conserved regions (SCR) common to the template and target. The alignment from ClustalW program was used to build the initial model. To construct a protein model for the target sequence, we used the InsightII/Homology program [35] implemented on a Silicon Graphics workstation. The initial model was improved by energy minimization. Ferric ion was added to the model in InsightII and it is ligated to three histidines, His525, His530, His716 and Ile864. Layers of water with thickness 5 Å were added to the whole protein using the SOAK program in the InsightII. A layer of water allows hydrophilic residues to interact with water instead of interacting with each other as they would do if modeled in vacuum. The protein model was energy minimized using CHARMm.cfrc [36] forcefield of CHARMM module in InsightII. The minimization was carried out using 300 steps of steepest descent followed by 500 steps of conjugate gradient to relieve all the bad contacts of the system. During the optimization procedure, the structure was verified by Profile-3D. The Profile-3D tests the validity of hypothetical protein structures by measuring the compatibility of the hypothetical structure with its own amino acid sequence [37]. To verify the protein model, the

coordinates of the protein model were submitted to PROCHECK [38] and ERRAT [39]. The stereo chemical quality of the protein structures is examined by a Ramachandran plot using the PROCHECK program, the number of residues that are in the allowed or disallowed regions of Ramachandran plot determines the quality of the model. The ERRAT program was used to assess the false statistics of bad non-bonded interactions within the structure model.

Energy minimization methods

GOLD (Genetic Optimization of Ligand Docking), a docking program that is based on genetic algorithm [40] is used to dock the compounds namely, nordihydroguaiaretic acid (NDGA) having IC_{50} of 1.5 μM [25], 1-(benzyloxy)hept-2-yn-4-ol having IC_{50} of 760 μM [26], 1-[(4methylbenzyl)oxy]hept-2-yn-4-ol having an IC_{50} of 45 μM [26] and [(hept-2-yn-1-ylsulfanyl)methyl]benzene) showing no inhibition [26] of the 5-LOX activity. The interactions of these four ligands with 5-LOX active site residues are thoroughly studied using the molecular mechanics calculations to understand the 5-LOX structure better and to get hints for better inhibitor design.

The parameters used for GA were population size (100), selection pressure (1.1), number of operations (100,000), number of islands (5) and niche size (2). Operator parameters for crossover, mutation and migration were set to 95, 95 and 10 respectively. Default cutoff values of 2.5 Å (d_{H-X}) for hydrogen bonds and 4.0 Å for Vander Waals were employed. During docking, the default algorithm speed was selected and the ligand binding site in the potato 5-LOX was defined within a 10 Å radius with the centroid as CE atom of His530. The number of poses for each inhibitor was set to 100, and early termination was allowed if the top three bound conformations of a ligand were within 1.5 Å RMSD. After docking, the individual binding poses of each ligand were observed and their interactions with the protein were studied. The best and most energetically favorable conformation of each ligand was selected.

Energy minimizations using the molecular mechanics calculations are useful to calculate the binding energies, but these methods met with only limited success initially [41], due to the large approximations involved in the analysis (e.g., solvent model used, lack of entropic terms, etc.). A few successful studies evaluated interaction energies and ligand strain in analysis of various binding conformations of new analogs. The purine nucleoside phosphorylase (PNP) inhibitors [42, 43] were designed successfully using these methods. Minimization methods were also used successfully, for predicting relative binding affinities of protease inhibitors [44], COX-2 inhibitors [45] and fructose 1–6, bisphosphatase inhibitors [46, 47] using energies obtained both in solvent as well as complex phases of each inhibitor.

In this article, the binding affinities of LOX inhibitors were estimated using energies obtained from minimization methods using molecular mechanics calculations. The binding modes of the inhibitors were obtained by carefully aligning docked structures of the inhibitors in the homology model of the LOX enzyme in the active site. These inhibitors are evaluated by performing minimization calculations both in solvent and in complex using the AMBER [48] force field. The following are the technical details used for estimating relative binding affinities using energy components obtained from minimizations of each inhibitor both in solvent as well as in complex phases.

Molecular mechanics calculations (energy minimizations) on all the structures were performed using the BORN module of the AMBER [48] program. A four-stage protocol was set up for energy minimizations of the protein-inhibitor complex. Minimization at each stage was performed using 100 steps of steepest descent and 1500 steps of conjugate gradient algorithms for minimization. In the first stage, only the water molecules were minimized, keeping the inhibitor and the protein (in the complex calculation) fixed. The purpose of this step is to relieve any bad contacts involving water molecules in the initially solvated system. In the second stage, only hydrogens in the system were allowed to relax. This step relaxes the hydrogen atoms prior to relaxing heavy atoms. It was performed because the hydrogen locations are not specified by the X-ray structure and because adjustments in hydrogen atom locations are necessary to improve hydrogen bond geometries. In this third stage, all atoms of the protein were fixed, while allowing all the atoms of the inhibitor and the solvent to move during optimization. This stage allows for the relaxation of the inhibitor with respect to the protein and establishes the preferred interactions (e.g., hydrogen bonds). In the fourth and final stage, all the atoms of residues within 25 Å from the center of the inhibitor (water molecules, protein atoms and the ligand) were allowed to relax.

A four-stage protocol was also established for energy minimization of the solvated inhibitor. These minimizations were carried out using periodic boundary conditions in all directions and each stage involved 100 steps of steepest descent and 1500 steps of conjugate gradient optimization. In the first stage of minimization, only the water molecules were minimized keeping the inhibitor (i.e., the solute) fixed. In the second stage, only hydrogens in the system were allowed to relax. In the third stage, all the atoms of the inhibitor were allowed to move while fixing the solvent during the optimization. In the fourth stage of the solvent calculation, all water molecules and all the atoms of the inhibitor were allowed to relax.

The minimized structures for all the inhibitors in the complexed and solvated states were used for calculating the following energy variables:

$$\Delta E_{\text{bind}}(\text{Intra}) = E_{\text{com}}(\text{Intra}) - E_{\text{sol}}(\text{Intra}) \quad (1)$$

$$\Delta E_{\text{bind}}(\text{Inter}) = E_{\text{com}}(\text{Inter}) - E_{\text{sol}}(\text{Inter}) \quad (2)$$

where, ΔE_{bind}(intra) and ΔE_{bind}(inter) are relative intra and intermolecular binding interaction energies of inhibitor, respectively, and E_{com}(Intra), E_{com}(Inter), E_{sol}(intra), and E_{sol}(Inter) are intra and intermolecular interaction energies of inhibitor in the complexed and solvated states, respectively. The total binding energy, ΔE_{bind} (Total), of inhibitor is given by,

$$\Delta E_{\text{bind}}(\text{Total}) = \Delta E_{\text{bind}}(\text{Intra}) + \Delta E_{\text{bind}}(\text{Inter}) \quad (3)$$

Results and discussion

There are many examples where homology modeling techniques have supported the drug discovery process especially in the target identification and/or validation, lead identification as well as lead optimization with respect to potency and selectivity. The extent of information derived from the homology model depends on the quality of the model. Since the accuracy of the homology model is related to the degree of sequence identity and similarity between the template and target, template search and sequence alignment are the crucial steps in any homology modeling. The BLASTP searches of PDB identified the crystal structure of Soybean Lipoxygenase (PDB ID: 1NO3) as the template structure.

A pair wise alignment of the sequences of the template and the target is presented in Fig. 1. The structurally conserved regions were determined by multiple sequence alignment, which is based on the Needleman and Wunsch Algorithm [49]. The model initially generated was energy minimized in CHARMM after addition of the Ferric ion. The final model obtained was verified with Profile-3D and Structure Alignment in InsightII. The overall self-compatibility score for this protein without the heteroatom is 342.899719 which is higher than the lower score 178.423093 and close to the top score 396.495761. The Profile-3D graph of the model is shown in Fig. 2.

The RMSD of the equivalent Cα atoms between the crystal structure 1NO3 and the final homology modeled structure of 5-LOX is 0.621534. The RMS values for all the non-hydrogen atoms between the crystal structure 1NO3 and the final homology modeled structure of 5-LOX within the 10 Å sphere of the active site is 0.558847, indicating that the overall structures are highly identical and that the homology model is reliable. The Cα trace of both the template and the model is shown in Fig. 3. The

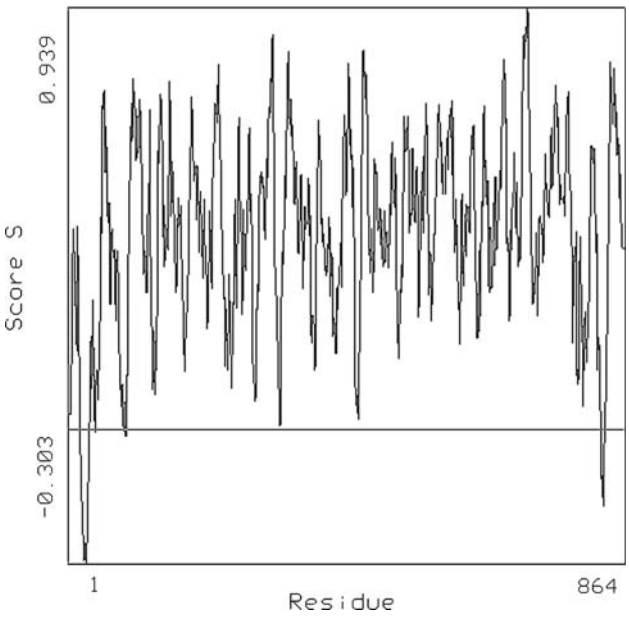


Fig. 2 Profile-3D graph of the model

overall secondary and tertiary structures of both the proteins are quite similar (Fig. 4). The stereochemical quality of the 5-LOX model was checked using the PROCHECK and ERRAT programs. Ramachandran plot of this minimized model showed that 99.3% of the residues were located in the allowed regions (82.4% most favored) and

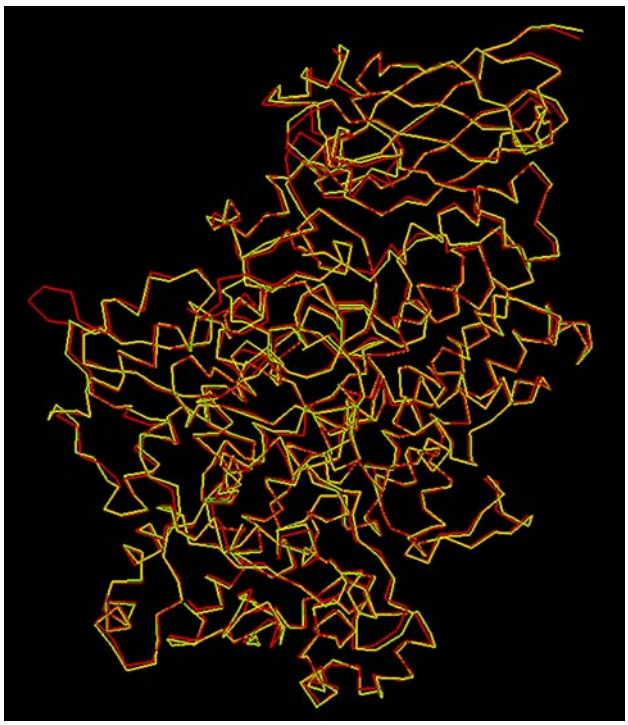


Fig. 3 Cα Trace of the template and the model. Yellow color shows the template and red color shows the model

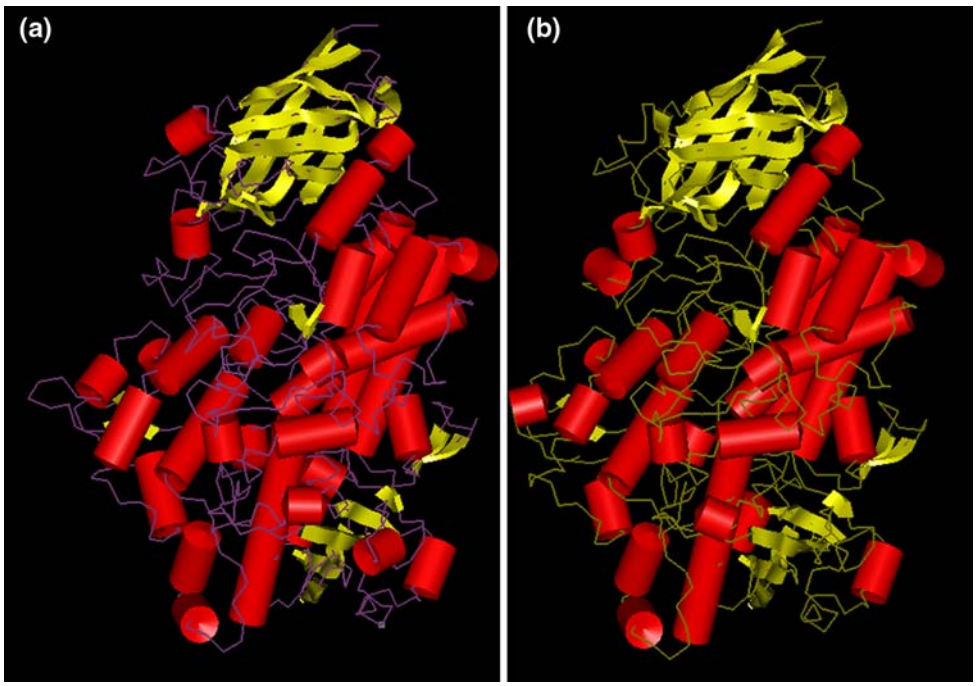


Fig. 4 Secondary structures (**a**) model and (**b**) template

only 0.7% (5 residues) outside the allowed regions. As these disallowed residues are far from the 5-LOX active site they do not significantly contribute to its function. The ERRAT results showed that the overall quality factor of the protein was good with a score of 88.318. Comparison of numerous LOX amino acid sequences as well as mutagenesis studies and crystal structure analysis led to the identification of highly conserved residues [50] and important residues, involved in iron chelation [51, 52]. The iron atom follows the structural pattern at the active site residues that is conserved in all LOXs. It is in distorted octahedral geometry. There are five amino acid ligands, the imidazole N atoms of three histidine residues His525, His530 and His716; carboxylate oxygen of the C-terminal isoleucine Ile864 and the carboxyl oxygen of the amide of an aspargine Asn720. The sixth coordination position faces an open cavity that usually accommodates the substrate/inhibitor. Fe–O distance with Ile864 is 2.10 Å. Fe-N distance with the Histidines 525, 530 and 716 is between 2.1 and 3 Å.

The overall folding pattern of lipoxygenases is conserved across the available 3D structures and is also recognized in the potato 5-LOX model. It is composed of two folding units; a small N-terminal β-barrel domain, suggested to interact with lipids and a larger C-terminal catalytic domain, mainly composed of α-helices, and containing the active site, i.e. iron-binding site and the substrate binding cleft. Based on the superimposition of soybean LOX-3 and potato 5-LOX 3D structures, active site was defined as a sphere of 10 Å centered on the inhibitor co-crystallized with soybean LOX-3. Polar residues like Gln521, Glu787, His530, His525, His271, His783, Thr784, Arg782, Asp276, Arg559, Asp560, Asn563, Asn565, Asn720, Ser567, Ser863 and Thr279 are distributed along the 5-LOX active site channel. Acidic amino acids (Asp276, Asp560) and basic amino acids (His525, His530) are present in the active site channel and play vital role in ligand binding. The knowledge of the orientation of these active site residues is extremely useful in designing a potential inhibitor of 5-LOX.

We used the GOLD program for the docking studies of nordihydroguaiaretic acid (NDGA) and analogs of benzyl propargyl ether in binding site of the 5-LOX structure. The interactions of NDGA with 5-LOX are studied using AMBER and Quanta [53]. It is observed that NDGA (**1**) is located in the center of the active site, and is stabilized by hydrogen bonding interactions as shown in Fig. 5a. In the molecule there are four hydroxyl moieties, each forming one or more hydrogen-bonding interactions with the active site amino acids. In the first hydroxyl group oxygen makes hydrogen bonding interactions with the side chain of His271 ($O^{\cdots}N\varepsilon = 2.75$ Å), and the hydrogen atom makes hydrogen-bonding interactions with the main chain of Asp276 ($OH^{\cdots}O = 2.4$ Å). In the second hydroxyl group, the hydrogen atom forms hydrogen bonding interactions with OD2 of Asp560 ($OH^{\cdots}OD2 = 2.72$ Å) and the oxygen atom forms two hydrogen bonds with amide group of Asn563 ($O^{\cdots}ND2 = 3.46$ Å). In the third hydroxyl group, hydrogen atom forms hydrogen bond with the main chain atom of Ile564 ($OH^{\cdots}O = 2.86$ Å) and oxygen atom forms two hydrogen bonds with Asn565 ($O^{\cdots}ND2 = 3.16$ Å). In the fourth hydroxyl group, hydrogen atom forms one hydrogen bond with His 530 ($OH^{\cdots}ND1 = 3.11$ Å). And

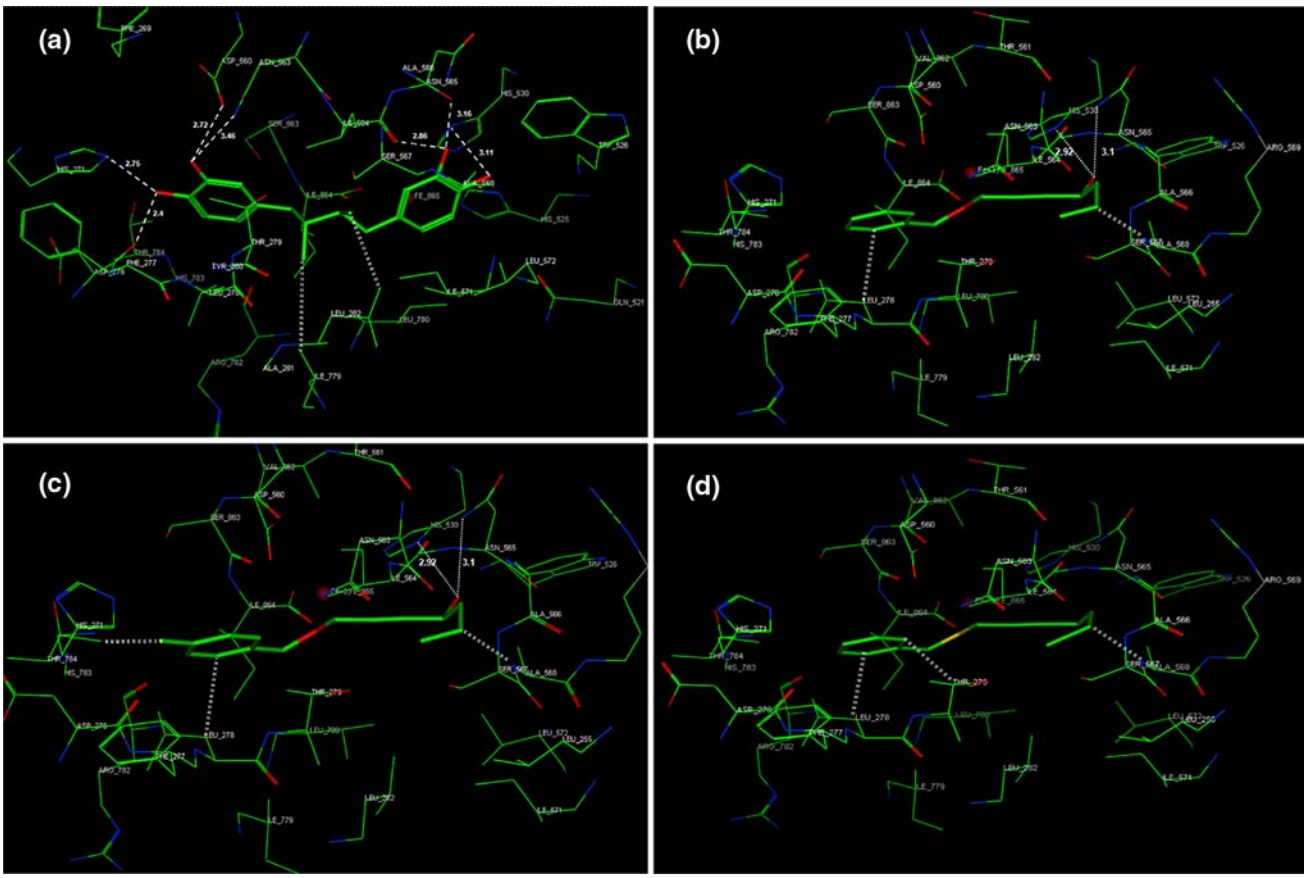
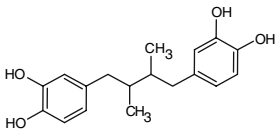
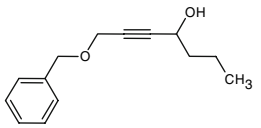
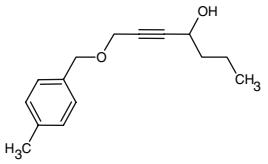
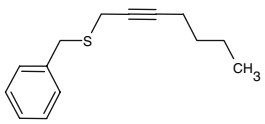


Fig. 5 Hydrogen bonding/hydrophobic interactions of the compounds with 5-LOX active site residues: (**a**) Compound **1**, (**b**) compound **2**, (**c**) compound **3** and (**d**) compound **4** (Hydrogen bonding interactions are shown as single dotted line and hydrophobic interactions as double dotted line)

also, NDGA picks-up hydrophobic interactions with Leu282, and Ile779 protein residues. In addition, NDGA is firmly bound in the open cavity that is in the sixth coordination of the iron atom and thus prevents the access of the substrate to the catalytic site of the enzyme efficiently. Hence, it has a very low IC_{50} and possesses high affinity. The second compound, 1-benzyloxy-2-heptyn-4-ol (**2**), formed three hydrogen bonds with 5-LOX (Fig. 5b). The hydroxyl group oxygen forms two hydrogen-bonding interactions with ND2 (via. HD1 and HD2) of Asn565 ($O^{\cdots}ND2 = 3.10$ Å). The hydrogen atom forms hydrogen bond with ND1 of His530 ($O–H^{\cdots}ND1 = 2.92$ Å). The third compound, 1-[(4methylbenzyl)oxy] hept-2-yn-4-ol (**3**), forms the same number of hydrogen bonding interactions as compound (**2**). In addition, the methyl group of this compound gains strong hydrophobic interactions with Thr784 (Fig. 5c). As a result the compound (**3**) is more potent than the compound (**2**) to 5-LOX. The fourth compound [(hept-2-yn-1-ylsulfanyl)methyl] benzene did not form any hydrogen bond interactions with the protein except a few hydrophobic interactions (Fig. 5d) with the protein residues. There are no strong interactions to stabilize the compound (**4**) in the 5-LOX active site, hence it cannot be a 5-LOX inhibitor. The residue Asn720 present in the 5-LOX active site may act as a ligand for iron and is a very important amino acid for the enzyme's activity. Based on the comparison of the number of hydrogen bonds formed between the compounds (**1**) and (**2**) with the 5-LOX indicates that the NDGA (**1**) is more potent inhibitor of 5-LOX than the 1-benzyloxy-2-heptyn-4-ol (**2**). The calculated binding energies using minimization methods and Eq. 3 (Table 1) for the compounds, **1**, **2**, **3**, and **4** with 5-LOX are, −25.5 kcal/mole, −11.6 kcal/mole, −21.4 kcal/mole and 10.5 kcal/mole, respectively which agree with the experimental results qualitatively (Table 1). Therefore, these binding energy results further validate the homology model of 5-LOX, which could be used for designing more potent inhibitors. The protein model presented here can also be used for designing inhibitors for human 5-LOX because of the high similarity among lipoxygenases in the catalytic domain containing conserved metal binding residues [54].

Table 1 Binding energies calculated using the Eq. 3 and the experimentally measured IC_{50} values for the compounds considered in the validation study

S. No.	Ligand name	Ligand structure	E_{bind} (Total) (kcal/mole)	Experimental IC_{50} (μM)	Reference
1	Nordihydroguaiaretic acid (NDGA)		−25.5	1.5	[25]
2	1-(benzyloxy)-2-heptyn-4-ol		−11.6	760	[26]
3	1-[(4methylbenzyl)-oxy]-2-heptyn-4-ol		−21.4	45	[26]
4	[(Hept-2-yn-1-ylsulfanyl) methyl]benzene (or) 1-(benzylsulfanyl)-2-heptyne		10.5	NI	[26]

Conclusions

The accuracy of a homology model built depends strongly on the degree of homology with the template sequence. The homology modeling methodology we used here for constructing the Potato 5-LOX model may be adequate because the model is based on a sequence homology of ~63% with the template. After energy minimization, the refined model structure obtained is assessed by Profile-3D and Procheck, and the results show that this model is reliable. However, to consider structural information about the target as well, a theoretical 3D model of 5-LOX was elaborated by homology with soybean lipoxygenases based on consensus alignment of the sequences. The 5-LOX active site was then characterized from a structural point of view and used to study the docking of selected inhibitors. This shed new light on the binding features of the enzyme. The active site pocket in the protein model constructed is conserved among all the lipoxygenases. The residue Asn720 forms the fifth coordination with iron, hence is very important residue. The accuracy of the protein model is reflected in docking and minimization studies with benzyl propargyl ether inhibitors. The minimization results correlated well with the activity measured for all the inhibitors which further validates the homology model. Finally, hydrogen bonds analysis identified the important hydrogen bonds between enzyme and inhibitors. Fundamental understanding of the molecular details of inhibitors/5-LOX interactions remains very rudimentary, in contrast with the extensive studies carried out on the localization and activation of the enzyme. The present study is a step towards the characterization of 5-LOX and its interaction with ligands. This model can be considered as a working tool for generating hypotheses and designing further experimental studies, and more precise predictions of function and binding affinities of inhibitors.

Acknowledgements This work was supported by research grants from Department of Biotechnology (DBT), Govt. of India (Grant # 37(1221)/05/EMR-II). We thank Centre for Modelling, Simulation and Design (CMSD), University of Hyderabad for permitting us to use the SGI workstation and InsightII facilities. We duly acknowledge Council of Scientific and Industrial Research (CSIR), Govt. of India for providing junior research fellowship to P. Aparoy.

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