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LOX1 inhibition with small molecules

Chrysoula Gousiadou [a,*], Irene Kouskoumvekaki [b]

[a] Department of Chemistry, Technical University of Denmark, DK-2800 Lyngby, Denmark
[b] Center for Biological Sequence Analysis, Department of Systems Biology, Technical University of Denmark, 2800 Lyngby, Denmark

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ABSTRACT

Lipoxygenases (LOXs) are nonheme, iron-containing dioxygenases that catalyze the dioxygenation of polyunsaturated fatty acids and are widely distributed among plant and animal species. Human LOXs, now identified as key enzymes in the pathogenesis of major disorders, have increasingly drawn the attention as targets and great effort has been made for the discovery and design of suitable inhibitors, to which end both pharmacological and computational methods have been employed. In the present work, using pharmacophore modeling and docking, we attempt to elucidate the inhibition of LOX1 with a new inhibitor, albidoside, an iridoid glucoside isolated from plants of the *Scutellaria* genus. Through a pharmacophore approach, complementarities between the ligand and the binding site are explored and a plausible mode of binding with the protein is suggested for albidoside.

1. Introduction

Lipoxygenases (LOXs) now identified as key enzymes in the pathogenesis of major human disorders [1–5], are a structurally related family of non-heme iron-containing dioxygenases that catalyze the addition of molecular oxygen to polyunsaturated fatty acids [6–9]. They are widely distributed in plants, fungi, invertebrates and mammals and recently they have been found in bacteria [10]. Essential structural features of both plant and animal LOXs are highly conserved and the topology of the catalytic domain is unique to the lipoxygenase family [11–19].

According to their structural features they are grouped into LOX1 and LOX2 gene families and they are classified according to their positional specificity of dioxygenation of most common substrates: linoleate (C-18) in plants, and arachidonic acid (C-20) in mammals. In plants 13- and 9-LOX1 and in mammals, 5-, 8-, 12-, 15-LOX1 and 15-LOX2 are known [6–9,11–19].

LOXs have a single polypeptide chain with a molecular mass of 75–81 kDa (≈662–711 amino acids) in mammals and 94–103 kDa (≈838–923 amino acids) in plants and are members of a multigene family exhibiting an overall sequence identity of ≈25–40%, while close functional homologues across species share 70–95% identity [10–19]. The tertiary structure which is the same in plant and mammalian LOXs reveals two domains: The *N*-terminal beta-barrel domain (Lipoxygenase homology domain) and a *C*-terminal domain which contains both the catalytically active nonheme iron and the substrate-binding cavity [11–19]. In a redox reaction involving the iron cofactor and molecular oxygen, they catalyse peroxidation of unsaturated fatty acids and co-oxidation of endobiotics and xenobiotics. The iron (Fe^{+2}) is ligated in an octahedral arrangement by 3 conserved histidines, one His/Asn/Ser, and the *C*-terminal isoleucine [11–19]. Studies of the various complexes of this enzyme with different inhibitors have found them in this location [12–15,17–19]. Finally, it has been reported that LOX1, when bound with an inhibitor, undergoes a conformational change. Two distinct conformations of the protein, the open (free) and closed (liganded) form have been described [20].

Both pharmacological and computational methods [21–23] have been employed for the discovery and design of suitable inhibitors, natural [1,24,25] or synthetic [26–29] molecules interacting strongly and specifically with the protein. LOX1 inhibitors are classified into reductive, chelative and competitive/mixed [24–29]. Many natural products are known reductive inhibitors – indicating the ease with which LOX1 isozymes can be inhibited in this manner – and are gaining attention as potential drugs or 'leads' for drug development [1,24,25]. Nevertheless, selectivity and specificity are demanded from all potential inhibitors. To date, 5-LOX-1 remains the only lipoxygenases enzyme for which there is an FDA-approved inhibitor (Zileuton) on the market [31].

In order to explore LOX1 inhibition, a Structure-Based Pharmacophore modeling has been attempted in the present work.

* Corresponding author.
E-mail address: chgou@kemi.dtu.dk (C. Gousiadou).

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Typically, this involves an analysis of the complementary chemical features of the binding site and the small molecule ligand, resulting in an assembly of selected features that comprise the pharmacophore model [32]. The model provides insights both on the binding interactions as well as the shape and volume of the active site. The pharmacophore features characterize a particular property and are not tied to a specific chemical structure [33]. Once generated and evaluated, the pharmacophore model was applied on a dataset of iridoid compounds, which, in a previous study, we investigated for *in vitro* inhibitory effect on soybean 13-LOX1 [34]. Iridoids are monoterpenes biosynthesized from isoprene and they exhibit a wide range of bioactivity [35]. They are natural products, often intermediating in the biosynthesis of alkaloids and typically found in plants as glucosides. Unlike other groups of natural products, generally known to be active against LOX1 (flavonoids as reductive inhibitors) [23,24], our iridoids exhibited negligible activity. Albidoside alone was a surprising case of discontinuous structure–activity relationship (SAR), inhibiting the protein with IC50 value of 62.5 μM [34]. Our pharmacophore model was in agreement with the *in vitro* results, selecting albidoside as the only compound out of the iridoid dataset as an inhibitor of LOX1 and identifying common pharmacophore features on its structure with known LOX1 inhibitors.

2. Methods

2.1. Docking simulations

Soybean 13-LOX1 (SBL1) has been the prototypical enzyme of its class and the best-studied model of lipoxygenases catalysis and structure [11]. SBL1 has been an excellent model system for understanding all lipoxygenases. It is relatively easy to purify, sufficiently stable and can be obtained in large quantities. In addition, the cDNA encoding soybean lipoxygenase has been cloned and sequenced and the protein has been expressed in bacterial systems. Many mechanistic and spectroscopic studies of lipoxygenases have been carried out with this enzyme: Many inhibitors and substrate analogs were also developed for this system [16]). Finally, in 1997 when the crystal structure of 15-LOX1 was reported, it was verified that the overall architecture of the mammalian lipoxygenase is similar to the soybean LOX1 enzyme although the mammalian structure is much more compact. The topology of the catalytic domain is unique to the lipoxygenase family as it is shared by no other protein structure except soybean lipoxygenase [17].

Therefore, for lack of crystal complexes of the soybean 13-LOX1 with an inhibitor and based on the evidence regarding the similarity of the overall architecture between the soybean 13-LOX1 and the mammalian 15-LOX1 [17], the 3D structure of the co-crystallized complex of the mammalian 15-LOX1 with the inhibitor RS7 ((2E)-3-(2-oct-1-yn-1-ylphenyl) acrylic acid, PDB id: 1LOX) has been considered [17] for the purposes of the present work. MOE 2011.10 was used for all docking simulations. An initial structure preparation was carried out, and the Protonate 3D option was used to determine ionization states and to add hydrogens. Atoms further apart than 8 Å from the binding site were fixed, heavy atoms in the binding site tethered with a weight of 5000 kcal/mol, water molecules farther than 4.5 Å from ligand or receptor were deleted and an initial refinement to an RMS (Root Mean Square) gradient of 0.1 kcal/mol/A was carried out by force field minimization. PFROSST [36] force field was selected, in which AMBER 10 parameters are used for macromolecules and parm@frosst parameters for small molecules. R-field was chosen as a solvation model, a choice made as a compromise between accuracy and speed. To this end, an implicit (continuum) solvation model was applied, meaning that in a dielectric medium the charge distribution of the solute polarizes the dielectric, thus inducing a Reaction Field in the solute cavity [37].

2.2. Pharmacophore generation

The pharmacophore queries were generated with the Pharmacophore Query Editor application in MOE 2011.10, using the Unified Annotation Scheme. The co-crystallized complex 1LOX was considered. All settings were identical to those described above.

2.3. Database search

1 A validation dataset of 42 active/inactive compounds (active = 30, inactive = 12) was created using MOE 2014.0901 (Table 1). All molecules were previously reported in literature and their activity/inactivity against LOX1 was well established [23–27,38–4]. Structural redundancy was avoided. The decoys (inactive) were similar to the active ligands on the basis of six physical descriptors (molecular weight, number of rotational bonds, hydrogen bond donors, hydrogen bond acceptors, calculated octanol-water partition coefficient (clogP) and polar surface area). All descriptors were calculated using the ChemBio3DUltra program. The chemical structures were imported to MOE as SDF files and were washed. Tautomeric and protonation states were enumerated. A conformational search with an RMSD (Root Mean Square Distance) limit up to 0.25 was carried out by using the LowModeMD method. Force field partial charges were calculated and a refinement to an RMS gradient of 0.005 kcal/mol/A was performed, resulting to a database of 5758 conformers.
2 A database containing 822 conformers of the iridoids previously tested [34] for activity against 13-LOX1 was created and the molecular descriptors were calculated, following the procedure described above (Table 2). The pKa value of the carboxylic acid group of albidoside was calculated with the method of prediction by atom typing (ChemBio3DUltra).

Database search was carried out with the Pharmacophore Search application in MOE 2014.0901. In order to be a hit, a molecule should match all features of the pharmacophore query.

3. Results and discussion

3.1. Self-docking of RS7 to 15-LOX1

As such an approach/work flow has not been reported previously for exploring LOX1 inhibition, its reliability would have to be established as a first step. The self-docking simulation of RS7 to 15-LOX1 reproduced both the placement of RS7 in the cavity and its interactions with the side chains of the protein with reliable accuracy (Fig. 1a and b).

For the top scoring conformer of RS7 (RMSD 0.5596) a scoring value of −9.5732 (Free Gibbs Energy) was estimated. The inhibitor can be seen placed with the aromatic ring deep in the hydrophobic pocket, while the terminal carboxylate and methyl groups are closely affiliated with more polar regions (Fig. 1a and b). In the ligand interactions diagram generated for the pose, the shapes of RS7 and the cavity of the receptor are complementary. The aromatic (hydrophobic) part of RS7 is depicted in close proximity to the "greasy" residues of the protein, whereas the terminal functional groups are exposed to solvent. The proximity contour is drawn loosely around the ligand, indicating more space availability in the receptor cavity. The molecule, depicted as an iron-bound ligand, adopts a U-shaped conformation in the pocket and is "squeezed" by the sidechains of hydrophobic amino acids. In the proximity of the "U" base is the catalytic iron held in place by histidines 361,