IN SILICO CHARACTERIZATION OF THE LOX-P PROTEIN THROUGH STRUCTURAL AND PHYLOGENETIC ANALYSIS

V.G. Vidhya[1]*, S. VijayaBharathi[1], G. Rubalakshmi[2], T. Uma Mageswari[1]

[1] Department of Biotechnology, Faculty of Science and Humanities, SRM Institute of Science and Technology, Kattankulathur - 603 203, Tamil Nadu, India

[2] GRD Bioclinical Research, Muthukalipatty, Rasipuram, Namakkal – 637401, Tamil Nadu India

Corresponding Author

V.G. Vidhya
Department of Biotechnology
Faculty of Science and Humanities
SRM Institute of Science and Technology
Kattankulathur – 603 203
Tamil Nadu, India

ABSTRACT:

This study presents an integrated in silico characterization of the LOX-P protein to better understand its structural, functional and evolutionary features as well as its potential relevance in interactions with natural bioactive compounds. The work includes sequence-based analysis, physicochemical profiling, prediction of secondary and tertiary structures, identification of transmembrane regions, multiple sequence alignment and phylogenetic assessment using established bioinformatics tools and databases. In addition, computational approaches were employed to examine the border molecular context of LOX-P and to support its preliminary evaluation as a candidate target for ligand interaction studies. By combining multiple levels of protein analysis within a single framework this study provides a systematic basis for further functional interpretation and future experimental investigation of LOX-P.

INTRODUCTION:

Lipoxygenase-related proteins are a group of biologically important proteins associated with lipid metabolism and related cellular functions. These proteins are generally involved in the oxidation of polyunsaturated fatty acids, leading to the formation of biologically active lipid derived molecules that participate in signaling, regulation and stress related responses. Because of their involvement in fundamental metabolic pathways, lipoxygenase associated proteins have attracted interest in studies related to structural biology, functional annotation and molecular interaction analysis. LOX-P, as a member within this protein family is therefore of interest for understanding how sequence composition and structural organization may influence its biological role.

The characterization of LOX-P through computational methods is particularly valuable when experimental structural information is limited or unavailable. Computational approaches allow researchers to investigate the primary sequence, physicochemical properties, conserved regions, predicted secondary and tertiary structures and evolutionary relationships of such proteins in a systematic manner. In addition, analysis such as transmembrane prediction, sequence alignment and phylogenetic comparison provide further insight into possible functional domains, localization patterns and conservation among related proteins. Together, these approaches help build a foundational understanding of LOX-P and support hypothesis generation for future biological studies.

The significance of this work lies in providing a comprehensive in silico framework for the preliminary characterization of LOX-P and its possible interaction with natural bioactive compounds. By combining structural, functional and phylogenetic analysis this study helps identify the potential relevance of LOX-P as a target for computational ligand screening. Such an approach is useful not only for understanding the biological importance of the protein but also for guiding future experimental validation, therapeutic exploration and natural compound-based search.

METHODOLOGY

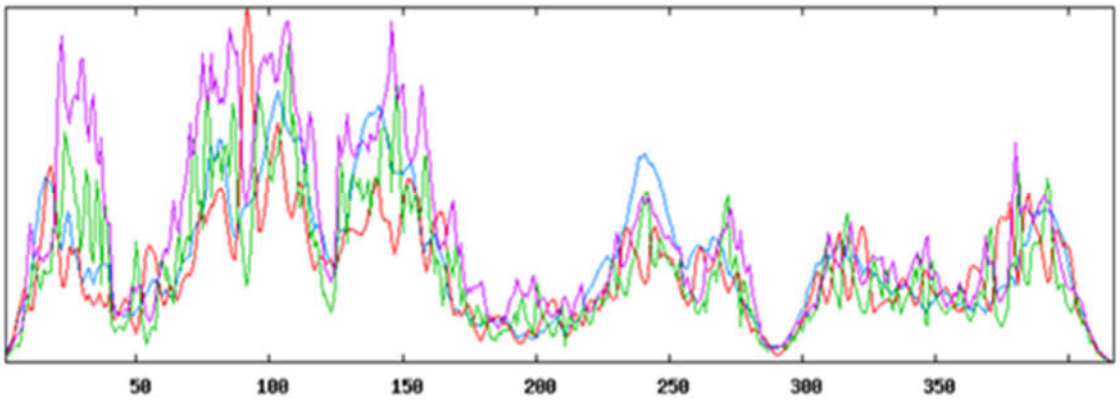
The protein sequence of LOX-P was retrieved from the uniprot database for subsequent physicochemical, structural and functional analysis. Amino acid composition, along with the distribution of hydrophilic and hydrophobic residues was analyzed using workbench. Key physiochemical properties including molecular weight, theoretical isoelectric point, extinction coefficient, instability index, aliphatic index and grand average of hydropathicity were computed. Conserved motifs within the protein sequence and functional features such as protein stability, occurrence and pattern of disulfide bonds were predicted. SOPMA was used to estimate the positional distribution of alpha helices, beta strands, turns and random coils using default parameters. For evolutionary analysis multiple sequence alignment and phylogenetic relationships were examined using the parsimony method with evolutionary divergence calculated by poisson method after executing gaps and missing data and the final phylogenetic tree was constructed using MEGA. Since the 3d structure of LOX-P structure prediction was done based on recognized fold domains.

RESULTS AND DISCUSSION

The protparm analysis provided the basic primary structural properties of the LOX-P protein. The results showed that the protein is composed of 453 amino acid and has a molecular weight of 48,736.70 Da indicating that it is a moderately sized protein. The total number of atoms present in the sequence was calculated as 6,767. Importantly the instability index classified the protein as unstable suggesting that it may have lower stability under in vitro conditions. These primary structural characteristics provide a basis for further physicochemical and functional analysis of the protein.

The secondary structure prediction indicated that the analyzed protein sequence with a length of 417 amino acids, is predominantly composed of random coils which account for 53..48 5 of the structure followed by alpha helices at 23.74 % extended beta strands at 16.31 % and beta turns at 6.47 % while 3_{10} helicesm pi helices, beta bridges, bend regions and ambiguous states were absent (Figure 1). This distribution suggests that the protein contains a high proportion of flexible and less ordered regions due to the dominance of random coils which may contribute to structural adaptability and functional interactions. At the same time the presence of a considerable proportion of alpha helices and beta strands indicated that the protein also possesses stable ordered regions essential for maintain its structural framework. Overall the results suggests that the protein has a mixed secondary structure with both stable folded elements and flexible regions which may be important for its biological activity.

Figure 1: Graphical representation showing the predicted distribution of secondary structural elements along the LOX-P protein sequence including alpha helices, beta strands, beta turns and random coils

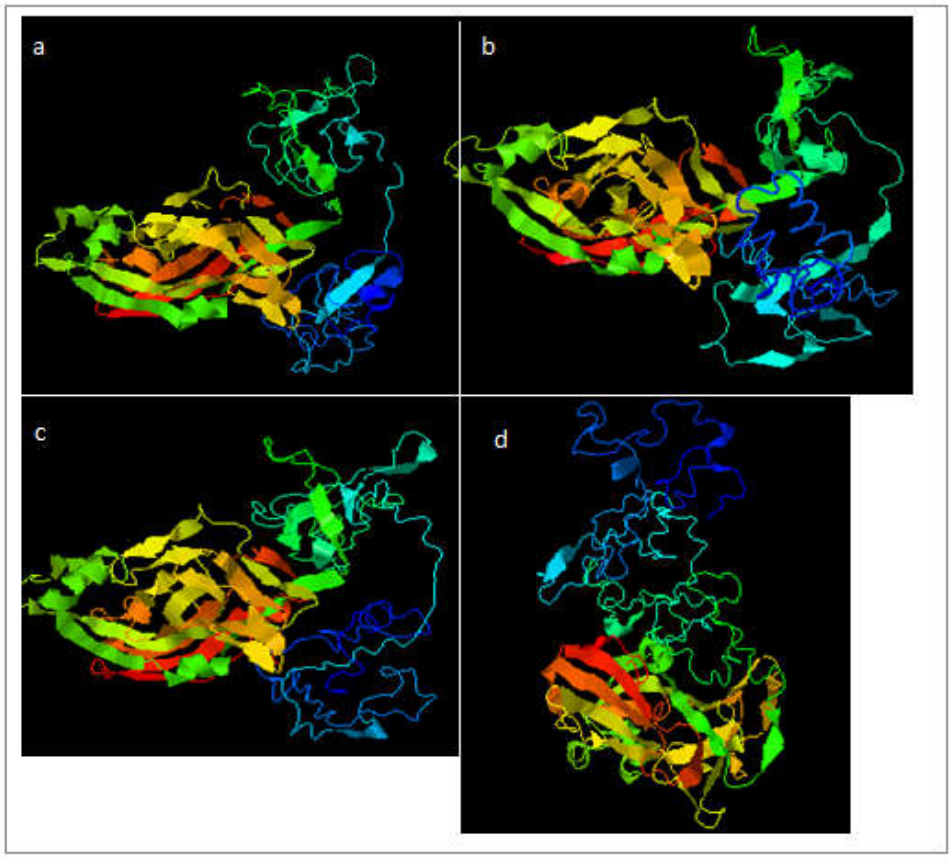


The domain based modelled structure results indicate the four possible three-dimensional models generated for the target protein with C-scores opf -0.44, -0.20, -2.22 and -3.55 (Figure 2). among these the model with a C-score of 0.20 can be considered the most reliable followed by the model with a c-score of -0.44 since higher C-scores indicate greater confidence in the predicted structure. The models with a C-scores of -2.22 and -3.55 show lower confidence and may be less accurate. The graphical models suggest that the protein contains both ordered folded regions and extended loop regions indicating a combination of stable structural domains and flexible segments. Thereby the predicted model provides a useful structural framework for the

protein and the top-ranked models may be used for further analysis such as functional interpretation, active site identification and docking studies.

Figure 2: Predicted 3D models of Lox-P protein (a-d represent the top four structural models ranked by confidence scores)

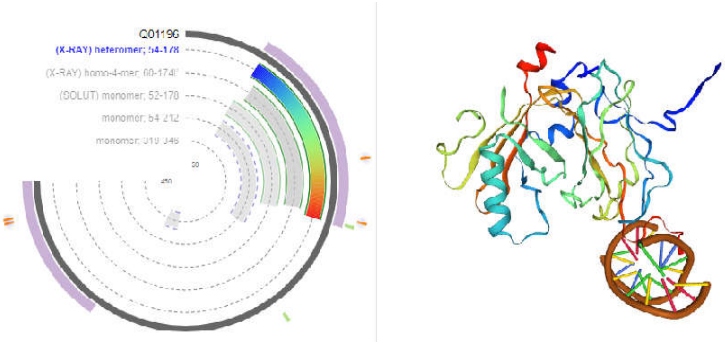
Model	C-score
Model 1	-0.44
Model 2	-0.20
Model 3	-2.22
Model 4	-3.55



The swiss-model analysis indicated that the target protein shows strong similarity to experimentally resolved core-binding factor subunits with several templated displaying very high sequence identity ranging from about 92 % to 100 %. The alignment with known structures such as Q01196 and Q13951 suggests that the query sequence is highly conserved in important functional regions supporting the reliability of homology-based model construction. Among the generated models, those with QMEAN values close to zero particularly the model with a QMEAN of 0.77 can be considered more reliable (Figure 3) and structurally acceptable whereas lower QMEAN values such as -4.66 indicate weaker model quality. The presence of multiple high

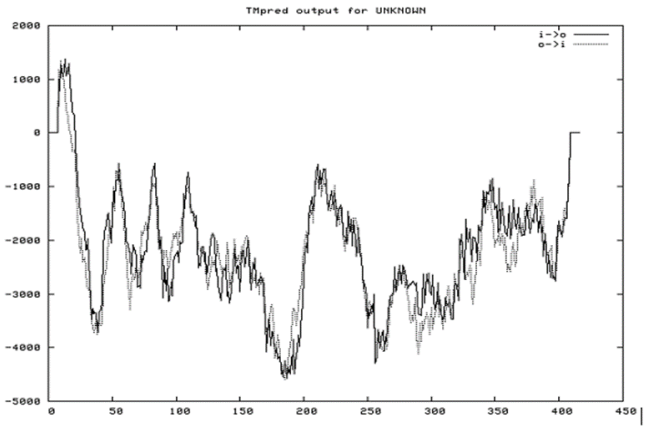
identity templates and acceptable quality scores suggests that the predicted 3d structure is dependable for further structural features with known core binding factor proteins and that the selected homology models are suitable for downstream analysis such as active site evaluation and molecular docking.

Figure 3: Modelled 3d structure of LOX-P protein



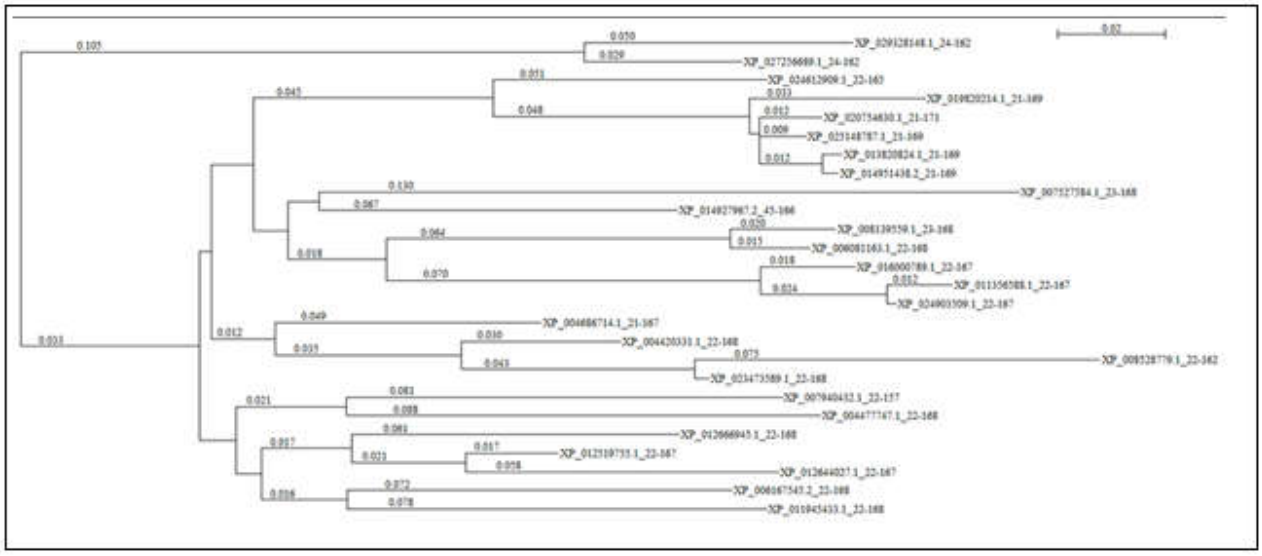
The TMPred analysis indicates that the Lox-P protein contains one significant transmembrane helix in the region spanning approximately residues 330 to 348 with a strongly preferred outside to inside orientation and a high score of 1023 while an alternative inside to outside model was predicted for residues 324 to 348 with a lower score of 770 (Figure 4). Since only scores above 500 are considered significant these predictions support the presence of a membrane -spanning segment but the outside to inside model is reliable. The suggested topology further indicate that that N-terminus is likely positioned outside the membrane supporting the preferred model. Overall, the results suggests that the LOX-P is likely a membrane associated protein containing a single strong transmembrane region which may play an important role in its localization, structural organization and biological function.

Figure 4: Graphical output showing the predicted transmembrane topology of the LOX-P protein



The LOX-P protein sequence was first subjected to BLAST analysis to identify homologous protein sequences from related organisms. The homologous sequences obtained from the BLAST search were then used for multiple sequence alignment which revealed several highly conserved amino acid residues and regions indicating strong structural and functional similarity among the selected proteins. A few variable regions and gaps were also observed reflecting limited sequence divergence among certain homologs. Based on the aligned sequences a phylogenetic tree was constructed (Figure 5) which grouped the LOX-P homologs into closely related clusters with relatively short branch lengths while some sequences appeared more distantly related. Overall, the BLAST, multiple sequence alignment and phylogenetic analysis suggest that the LOX-P protein is highly conserved and shares a close evolutionary relationship with related homologous proteins.

Figure 5: Phylogenetic tree showing the evolutionary relationship of LOX-P protein with its homologous sequences



CONCLUSION

The present *in silico* study provided a comprehensive characterization of the LOX-P protein through sequence, structural and phylogenetic analysis. The results offered useful insights into its physicochemical properties, secondary and tertiary structural features and evolutionary relationship with related proteins. The predicted 3D model and validation parameters suggested that the protein possesses a stable and reliable structural framework for further biological interpretation. Phylogenetic analysis also supported its conserved relationship among homologous sequences indicating possible functional significance. Thus this study demonstrates that the computational approaches are affective for understanding the structural and evolutionary aspects of LOX-P protein and can serve as a foundation for further experimental validation and functional studies.

REFERENCES

1. Giordano D, et al. Structural and Functional Characterization of Lipoxygenases from Diatoms by Bioinformatics and Modelling Studies. *Biomolecules.* 2024.
2. Kaur Y, et al. Molecular, in silico and expression studies on lipoxygenases in potato. *Plant Cell Reports* / PubMed-listed 2023 article.
3. Xu L, et al. A genome-wide study of the lipoxygenase gene families in *Medicago truncatula* and *Medicago sativa* reveals that MtLOX24 participates in the methyl jasmonate response. *BMC Plant Biology.* 2024.
4. Hao H, et al. Genome-wide identification and characterization of the lipoxygenase gene family in *Luffa aegyptiaca*. *Scientific Reports.* 2025.
5. Zhu Z, et al. Intricate Evolution of Multifunctional Lipoxygenase in Red Algae. 2024.
6. Parvez S, et al. Computational insights into the targeted inhibition of lipoxygenase in *Pseudomonas aeruginosa*: hints for drug design. *Archives of Microbiology.* 2025.
7. Metuge JA, et al. Effects of some anti-ulcer and anti-inflammatory natural products on cyclooxygenase and lipoxygenase enzymes: insights from in silico analysis. *Inflammopharmacology.* 2024.
8. Iqbal A, et al. Multimodal evaluation of lipoxygenase-targeting NSAIDs supported by in vitro and in silico studies. 2025.

9. Patel E, et al. Mutation in soybean Lox-2 PLAT/LH2 domain through computational approaches. 2025.
10. Lamlom SF, et al. Genome-wide identification reveals expansion and stress-responsive expression of the lipoxygenase gene family in soybean. *Frontiers in Plant Science.* 2025. Useful for LOX family background, conserved motifs, and phylogenetic framing.
11. Kim JW, et al. Discovery and molecular characterization of a novel 9S-lipoxygenase from *Enhygromyxa salina* for fatty acid biotransformations. *Journal of Agricultural and Food Chemistry.* 2024.
12. Viviani LG, et al. Identification of novel human 15-lipoxygenase-2 inhibitors using a virtual screening approach. *Journal of Medicinal Chemistry.* 2025.